ir Quality Criteria for Particulate Matter, Volume I of II</title><secondary-title>Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina</secondary-title></titles><periodical><full-title>Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina</full-title></periodical><pages>900, http://ofmpub.epa.gov/eims/eimscomm.getfile?p_download_id=435945</pages><volume>EPA/600/P-99/002aF</volume><dates><year>2004</year></dates><urls></urls></record></Cite></EndNote>]. In comparison, occupational health organizations rely on unified size fraction definitions based on the upper size cuts of particles and entry into the different regions of the respiratory tract. For example, the American Conference of Governmental Industrial Hygienists (ACGIH) considers 10 μm D_a particles as an upper limit for particles with this size entering the alveolar region [ADDIN EN.CITE <EndNote><Cite><Author>ACGIH</Author><Year>1999</Year><RecNum>52</RecNum><DisplayText>[24]</DisplayText><record><rec-number>52</rec-number><foreign-keys><key app="EN" db-id="xs0a90va7aasfwex5aev0dvyp0t59sta5dae" timestamp="1595791048">52</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>ACGIH</author></authors></contributors><titles><title>Particle Size-Selective Sampling for Health-Related Aerosols</title><secondary-title>American Conference of Governmental Industrial Hygienists, Air Sampling Procedures Committee, Ed. Vincent, J.H.</secondary-title></titles><periodical><full-title>American Conference of Governmental Industrial Hygienists, Air Sampling Procedures Committee, Ed. Vincent, J.H.</full-title></periodical><pages>240,

https://www.acgih.org/forms/store/ProductFormPublic/particle-size-selective-sampling-for-particulate-air-contaminants</pages><volume>ISBN 1-1882417-30-5</volume><dates><year>1999</year></dates><urls></urls></record></Cite></EndNote>]. Further, consideration must also be given to particle settling that may occur. For example, in still air, 10 µm spherical particles with a density of 1 g/cm^3 can remain airborne for approximately 8 minutes [ADDIN EN.CITE <EndNote><Cite><Author>Baron</Author><Year>2004</Year><RecNum>53</RecNum><DisplayText>[25]</DisplayText><record><rec-number>53</rec-number><foreign-keys><key app="EN" db-id="xs0a90va7aasfwex5aev0dvyp0t59sta5dae" timestamp="1595791478">53</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Baron, P.</author></authors></contributors><titles><title>Generation and Behavior of Airborne Particles (Aerosols)</title><secondary-title>Division of Applied Technology, National Institute for Occupational Safety and Health, Centers for Disease Control and Prevention</secondary-title></titles><periodical><full-title>Division of Applied Technology, National Institute for Occupational Safety and Health, Centers for Disease Control and Prevention</full-title></periodical><pages>40, https://www.cdc.gov/niosh/topics/aerosols/pdfs/aerosol_101.pdf</pages><dates><year>2004</year></dates><urls></urls></record></Cite></EndNote>]. However, as particle size decreases, the airborne settling time increases (*e.g.*, approximately 1.5 hours for 3 µm particles to settle in still air) [ADDIN EN.CITE <EndNote><Cite><Author>Baron</Author><Year>2004</Year><RecNum>53</RecNum><DisplayText>[24, 25]</DisplayText><record><rec-number>53</rec-number><foreign-keys><key

app="EN" db-id="xs0a90va7aasfwex5aev0dvyp0t59sta5dae" timestamp="1595791478">53</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Baron, P.</author></authors></contributors><titles><title>Generation and Behavior of Airborne Particles (Aerosols)</title><secondary-title>Division of Applied Technology, National Institute for Occupational Safety and Health, Centers for Disease Control and Prevention</secondary-title></titles><periodical><full-title>Division of Applied Technology, National Institute for Occupational Safety and Health, Centers for Disease Control and Prevention</full-title></periodical><pages>40, https://www.cdc.gov/niosh/topics/aerosols/pdfs/aerosol_101.pdf</pages><dates><year>2004</year></dates><urls></urls></record></Cite><Cite><Author>ACGIH</Author><Year>1999</Year><RecNum>52</RecNum><record><rec-number>52</rec-number><foreign-keys><key app="EN" db-id="xs0a90va7aasfwex5aev0dvyp0t59sta5dae" timestamp="1595791048">52</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>ACGIH</author></authors></contributors><titles><title>Particle Size-Selective Sampling for Health-Related Aerosols</title><secondary-title>American Conference of Governmental Industrial Hygienists, Air Sampling Procedures Committee, Ed. Vincent, J.H.</secondary-title></titles><periodical><full-title>American Conference of Governmental Industrial Hygienists, Air Sampling Procedures Committee, Ed. Vincent, J.H.</full-title></periodical><pages>240, https://www.acgih.org/forms/store/ProductFormPublic/particle-size-selective-sampling-for-particulate-air-contaminants</pages><volume>ISBN 1-1882417-30-5</volume><dates><year>1999</year></dates><urls></urls></record></Cite></EndNote>].

Therefore, solids with even a small fraction of respirable particles may produce prolonged and elevated airborne levels of respirable particles in the workplace. Though occupational monitoring data provide the most direct assurance that airborne levels of respirable particles do not exceed relevant exposure limits, particle size distribution data are typically the only metric available for estimating potential respirability for new chemical substances. Given this limitation and the reality that nearly all solid particulate materials may contain some percentage of respirable particles, a practical screening cutoff is warranted for category inclusion/exclusion. For the purposes of this category, we propose that HMW polymers are considered respirable if they are manufactured, processed, used, *etc*., in a manner that generates the new chemical substance with a particle or aerosol size of less than or equal to 10 µm or if respirable particles may be unintentionally generated during the life cycle of the material (*e.g.*, impaction and friction during transport). Under the latter scenarios, a practical cutoff of $\geq$ 1% respirable particles by weight (wt%) as the cutoff for assessing respirable particles and this percentage would be based on particle size distribution data for the material. The practical cutoff of $\geq$ 1 wt% is the same cutoff EPA applies to the nonreportable content of nanoscale materials [ADDIN EN.CITE <EndNote><Cite><Author>EPA</Author><Year>2017</Year><RecNum>54</RecNum><DisplayText>[26]</DisplayText><record><rec-number>54</rec-number><foreign-keys><key app="EN" db-id="xs0a90va7aasfwex5aev0dvyp0t59sta5dae" timestamp="1595791830">54</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>Chemical Substances When Manufactured or Processed as Nanoscale Materials; TSCA Reporting and Recordkeeping Requirements</title><secondary-title>Federal Register</secondary-title></titles><periodical><full-title>Federal Register</full-title></periodical><pages>3641-

3655</pages><volume>82</volume><number>8</number><dates><year>2017</year></dates><urls></urls></record></Cite></EndNote>]. This same cutoff would apply to the particle/droplet size distribution in the case of aerosols of a solid or liquid chemical substance and would be determined based on droplet size data for the material and/or liquid application method (*e.g.*, spray, aerosol, mist).

EPA's FG and FGEW criteria for E1 polymers provide a starting point for evaluating the potential reactivity and/or cytotoxicity of HMW polymers. Therefore, we propose using these criteria as an initial screen for determining whether a HMW polymer is considered non-reactive or reactive and included or excluded from the category, respectively. As shown in [REF _Ref46665925 \h * MERGEFORMAT], the E1 polymer exemption criteria include low-concern, moderate-concern, or high-concern FGs. A summary of representative FGs meeting each of these hazard concern levels is shown in [REF _Ref46674358 \h * MERGEFORMAT].

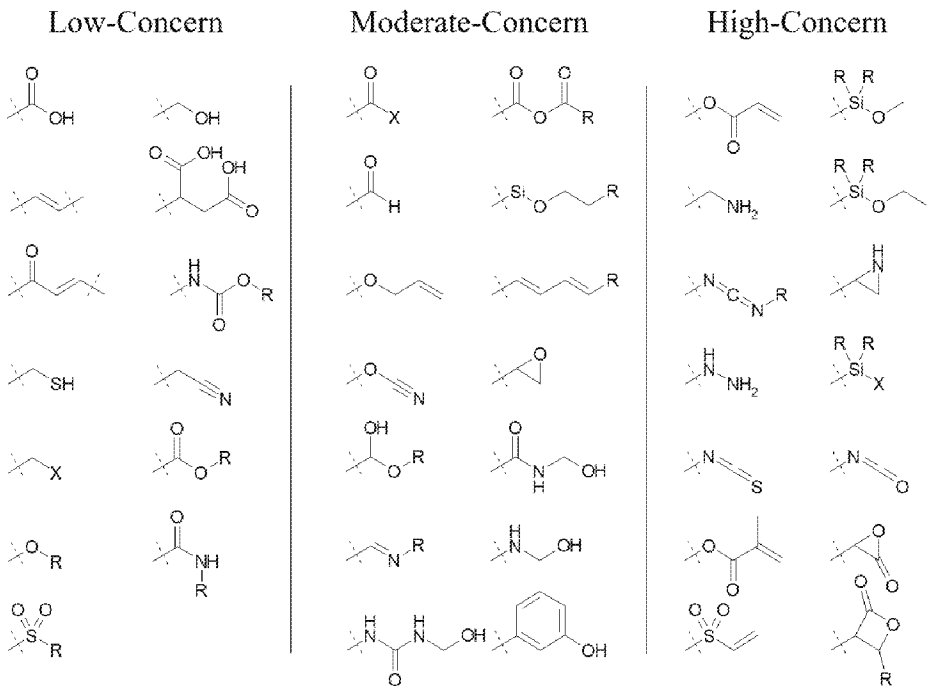


Figure [SEQ Figure * ARABIC]. FG hazard concern levels for polymeric substances meeting EPA's E1 polymer exemption criteria. The FGs shown above are representative alerts for identifying a HMW polymer as non-reactive (low concern)/reactive (moderate or high concern) for the HMW polymer category. The following cutoffs are proposed as the category boundaries for establishing that a HMW polymer is non-reactive: low-concern FGs (no limit), moderate-concern FGs (FGEW $\geq$ 1,000), or high-concern FGs (FGEW $\geq$ 5,000). "R" represents an undefined structure; "X" represents a halide. See: EPA (1997) [ADDIN EN.CITE <EndNote><Cite><Author>EPA</Author><Year>1997</Year><RecNum>36</RecNum><DisplayText>[5]</DisplayText><record><rec-number>36</rec-number><foreign-keys><key app="EN" db-id="xs0a90va7aasfwex5aev0dvyp0t59sta5dae"

[PAGE]

timestamp="1595771575">36</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>Polymer Exemption Guidance Manual</title><secondary-title>Office of Pollution Prevention and Toxics, U.S. Environmental Protection Agency, 1200 Pennsylvania Ave., NW, Washington, DC 20460</secondary-title></titles><periodical><full-title>Office of Pollution Prevention and Toxics, U.S. Environmental Protection Agency, 1200 Pennsylvania Ave., NW, Washington, DC 20460</full-title></periodical><pages>54, https://www.epa.gov/sites/production/files/2015-03/documents/polyguid.pdf</pages><volume>EPA 744-B-97-001</volume><dates><year>1997</year></dates><urls></urls></record></Cite></EndNote>] for further details.

A generally recognized property of respirable, low reactive (*i.e.*, low toxicity) particles that can cause lung overload is the poorly soluble nature of these compounds. EPA has published general water solubility classifications, which include: negligible solubility (*i.e.*, < 0.1 mg/L), slight solubility (*i.e.*, > 0.1 - 100 mg/L), moderate solubility (*i.e.*, > 100 - 1,000 mg/L), soluble (> 1,000 - 10,000 mg/L), and very soluble (> 10,000 mg/L) [ADDIN EN.CITE <EndNote><Cite><Author>EPA</Author><Year>2012</Year><RecNum>56</RecNum><DisplayText>[27]</DisplayText><record><rec-number>56</rec-number><foreign-keys><key app="EN" db-id="xs0a90va7aasfwex5aev0dvyp0t59sta5dae" timestamp="1595792442">56</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>Section 5. Estimating Physical/Chemical and Environmental Fate Properties with EPI SuiteTM, Sustainable Futures/P2 Framework Manual</title><secondary-title>Office of Pollution

Prevention and Toxics, U.S. Environmental Protection Agency, 1200 Pennsylvania Ave., NW, Washington, DC 20460</secondary-title></titles><periodical><full-title>Office of Pollution Prevention and Toxics, U.S. Environmental Protection Agency, 1200 Pennsylvania Ave., NW, Washington, DC 20460</full-title></periodical><pages>22, https://www.epa.gov/sites/production/files/2015-05/documents/05.pdf</pages><volume>EPA-748-B12-001</volume><dates><year>2012</year></dates><urls></urls></record></Cite></EndNote>]. These values were not established for evaluating the solubility of particles for lung overload; however, they may be used as conservative cutoffs for extractability, per OECD TG 120 [ADDIN EN.CITE <EndNote><Cite><Author>OECD</Author><Year>2000</Year><RecNum>55</RecNum><DisplayText>[28]</DisplayText><record><rec-number>55</rec-number><foreign-keys><key app="EN" db-id="xs0a90va7aasfwex5aev0dvyp0t59sta5dae" timestamp="1595792078">55</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>OECD</author></authors></contributors><titles><title>Solution/Extraction Behaviour of Polymers in Water</title><secondary-title>OECD Guideline for Testing of Chemicals</secondary-title></titles><periodical><full-title>OECD Guideline for Testing of Chemicals</full-title></periodical><pages>4, https://www.oecd-ilibrary.org/environment/oecd-guidelines-for-the-testing-of-chemicals-section-1-physical-chemical-properties_20745753</pages><volume>120</volume><dates><year>2000</year></dates><urls></urls></record></Cite></EndNote>], for measuring the insolubility/solubility of HMW polymers. ECETOC (2013) [ADDIN EN.CITE

<EndNote><Cite><Author>ECETOC</Author><Year>2013</Year><RecNum>9</RecNum><DisplayText>[29]</DisplayText><record><rec-number>9</rec-number><foreign-keys><key app="EN" db-id="xs0a90va7aasfwex5aev0dvyp0t59sta5dae" timestamp="1590845309">9</key></foreign-keys><ref-type name="Report">27</ref-type><contributors><authors><author>ECETOC</author></authors></contributors><titles><title>Poorly Soluble Particles / Lung Overload</title></titles><pages>130, http://www.ecetoc.org/wp-content/uploads/2014/08/ECETOC-TR-122-Poorly-Soluble-Particles-Lung-Overload.pdf</pages><number>Technical Report No. 122</number><dates><year>2013</year><pub-dates><date>December 2013</date></pub-dates></dates><pub-location>Brussels, Belguim</pub-location><publisher>European Centre for Ecotoxicology and Toxicology of Chemicals</publisher><work-type>Technical Report</work-type><urls><related-urls><url>http://www.ecetoc.org/wp-content/uploads/2014/08/ECETOC-TR-122-Poorly-Soluble-Particles-Lung-Overload.pdf</url></related-urls></urls></record></Cite></EndNote>] proposed an initial biosolubility screening approach that provided qualitative determinants (*i.e.*, "soluble", "insoluble", "Low dissolution rate", or "Very high dissolution rate") for assessing biosolubility; however, no quantitative thresholds were provided. In comparison, the International Commission on Radiological Protection (ICRP) and the German Federal Institute for Occupational Safety and Health (FIOSH) provide quantitative biosolubility cutoffs. ICRP describes three categories of soluble radiological materials: Fast (all material rapidly dissolves at a rate of 100 day^{-1}), Moderate (10% of the material dissolves rapidly and the rest dissolves at a rate of 0.005 day^{-1}), and Slow (0.1% of the material dissolves rapidly and the rest dissolves at a rate of 0.0001 day^{-1}) [ADDIN EN.CITE

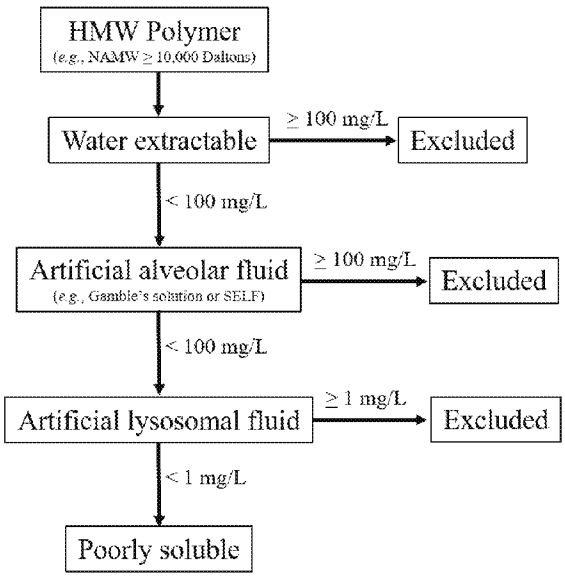
<EndNote><Cite><Author>ICRP</Author><Year>1994</Year><RecNum>26</RecNum><Dis
playText>[20]</DisplayText><record><rec-number>26</rec-number><foreign-keys><key
app="EN" db-id="xs0a90va7aasfwex5aev0dvyp0t59sta5dae"
timestamp="1590848620">26</key></foreign-keys><ref-type name="Journal Article">17</ref-
type><contributors><authors><author>ICRP</author></authors></contributors><titles><title>
Human respiratory tract model for radiological protection. A report of a Task Group of the
International Commission on Radiological Protection</title><secondary-title>Ann
ICRP</secondary-title><alt-title>Annals of the ICRP</alt-title></titles><periodical><full-
title>Ann ICRP</full-title><abbr-1>Annals of the ICRP</abbr-1></periodical><alt-
periodical><full-title>Ann ICRP</full-title><abbr-1>Annals of the ICRP</abbr-1></alt-
periodical><pages>1-482</pages><volume>24</volume><number>1-
3</number><edition>1994/01/01</edition><keywords><keyword>Humans</keyword><keywo
rd>International Cooperation</keyword><keyword>*Models,
Theoretical</keyword><keyword>Neoplasms, Radiation-
Induced/*etiology/pathology/physiopathology</keyword><keyword>Radiation
Dosage</keyword><keyword>*Radiation Monitoring</keyword><keyword>*Radiation
Protection</keyword><keyword>Radioactive Pollutants</keyword><keyword>Respiratory
System/pathology/physiopathology/*radiation effects</keyword><keyword>Respiratory Tract
Neoplasms/*etiology/pathology/physiopathology</keyword></keywords><dates><year>1994</
year></dates><isbn>0146-6453 (Print)
0146-6453</isbn><accession-
num>7726471</accession-num><urls><related-
urls><url>https://journals.sagepub.com/doi/pdf/10.1177/ANIB_24_1-3</url></related-
urls></urls><remote-database-provider>NLM</remote-database-

provider><language>eng</language></record></Cite></EndNote>]. FIOSH proposed a simulated solubility threshold of ≤ 1 mg/L in artificial lung fluids for identifying particles as "low soluble dusts" [ADDIN EN.CITE <EndNote><Cite><Author>BAUA</Author><Year>2017</Year><RecNum>57</RecNum><DisplayText>[30]</DisplayText><record><rec-number>57</rec-number><foreign-keys><key app="EN" db-id="xs0a90va7aasfwex5aev0dvyp0t59sta5dae" timestamp="1595794599">57</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>BAUA</author></authors></contributors><titles><title>Methodology for the Identification of Granular Biopersistent Particles (GBP) at Workplaces</title><secondary-title>Federal Institute for Occupational Safety and Health</secondary-title></titles><periodical><full-title>Federal Institute for Occupational Safety and Health</full-title></periodical><pages>103, https://www.baua.de/EN/Service/Publications/Report/F2336.pdf</pages><dates><year>2017</year></dates><urls></urls></record></Cite></EndNote>].

As discussed previously, the screening particle size cutoff and percentage of respirable particles for inclusion in this HMW polymer category are ≤ 10 µm and ≥ 1 wt%, respectively. These criteria are readily determinable based on the intended use and life cycle of the HMW polymer. However, determining whether a HMW polymer is "poorly soluble" and a potential hazard concern for lung overload is also dependent on the potential daily exposure estimates. Therefore, we propose using the inclusion/exclusion cutoffs shown in [REF _Ref46673847 \h * MERGEFORMAT], which consider water extractability/biosolubility and the legally binding permissible exposure limit (PEL), as mandated by the U.S. Occupational Safety and Health

Administration (OSHA) for respirable particulates not otherwise regulated or PNOR (*i.e.*, 5 mg/m^3).

Scheme [SEQ Scheme * ARABIC]. Screening criteria for determining water extractability and biosolubility.



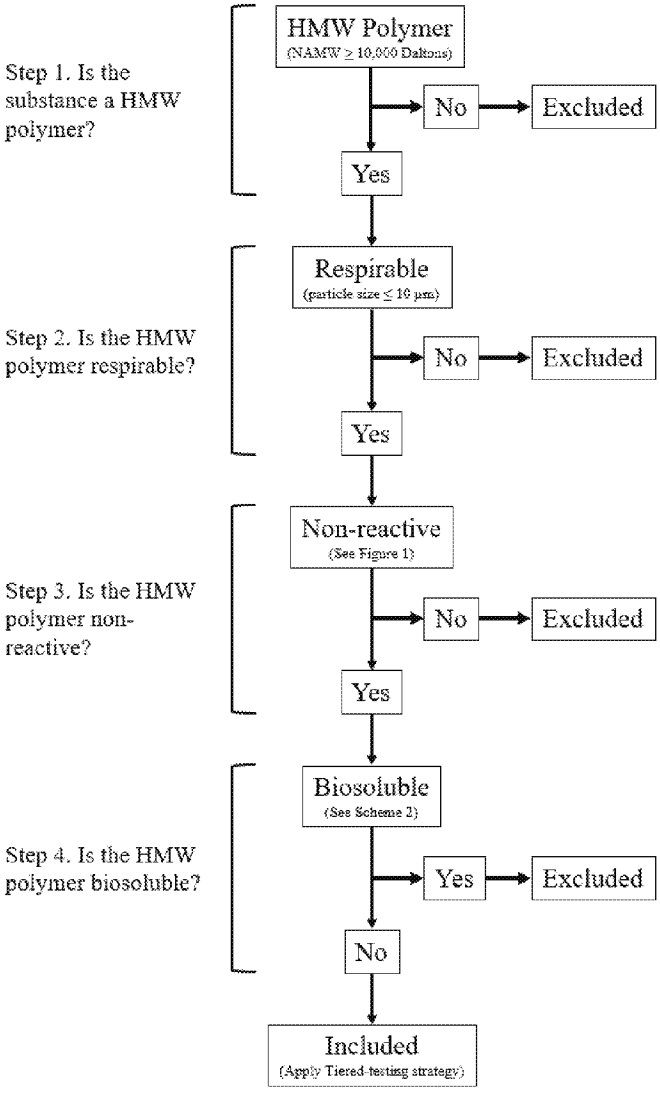
The proposed cutoffs shown in Scheme 1 are based on the following considerations. The first screen is water extractability using the cutoff for moderately water-soluble substances. While the screen is intended to identify insoluble (*i.e.*, non-extractable) HMW polymers, the EPA water solubility classifications were not specifically established to identify potential hazards related to lung overload and have not been established to correlate with biosolubility or biopersistence. Therefore, EPA's cutoff for moderate water solubility (*i.e.*, 100 mg/L) was selected rather than

the low water solubility cutoff, since it represents a transition from slight to moderate water solubility and is therefore expected to be conservatively inclusive in the first step because water extractability is generally expected to overestimate the insolubility of polymers in biological fluids. In the second screen, two biosolubility cutoffs may be used, either 100 mg/L or 1 mg/L, depending on the test system used (*e.g.*, simulated epithelial lung fluid or artificial alveolar macrophage lysosomal fluid). These values account for the biosolubility of the HMW polymer, as well as the OSHA PNOR PEL of 5 mg/m^3 (*i.e.*, 50 mg/day; 5 mg/m^3 × 10 m^3/day) for the respirable fraction. The first value is based on EPA (2020) [ADDIN EN.CITE <EndNote><Cite><Author>EPA</Author><Year>2020</Year><RecNum>75</RecNum><DisplayText>[31]</DisplayText><record><rec-number>75</rec-number><foreign-keys><key app="EN" db-id="xs0a90va7aasfwex5aev0dvyp0t59sta5dae" timestamp="1595843741">75</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>Revocation of Significant New Use Rule for a Certain Chemical Substance (P-16-581), Proposed rule</title><secondary-title>Federal Register</secondary-title></titles><periodical><full-title>Federal Register</full-title></periodical><pages>18179-18181</pages><volume>85</volume><number>63</number><dates><year>2020</year></dates><urls></urls></record></Cite></EndNote>], where the Agency applied a biosolubility cutoff of approximately 100 mg/L/day for a polymer in simulated epithelial lung fluid. This value would equate to a mean dissolution rate of approximately 72 mg/day in humans, based on an estimated daily alveolar fluid turnover of 0.72 L [ADDIN EN.CITE <EndNote><Cite><Author>Fronius</Author><Year>2012</Year><RecNum>58</RecNum><DisplayText>[32]</DisplayText><record><rec-number>58</rec-number><foreign-keys><key

app="EN" db-id="xs0a90va7aasfwex5aev0dvyp0t59sta5dae" timestamp="1595795295">58</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Fronius, M.</author><author>Clauss, W.G.</author><author>Althaus, M.</author></authors></contributors><titles><title>Why do we have to move fluid to be able to breath?</title><secondary-title>Frontiers in Physiology</secondary-title></titles><periodical><full-title>Frontiers in Physiology</full-title></periodical><pages>5, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3357553/pdf/fphys-03-00146.pdf</pages><volume>3</volume><dates><year>2012</year></dates><urls></urls></record></Cite></EndNote>]. The second value is based on the German FIOSH biosolubility cutoff of 1 mg/L for granular biopersistent particles. We propose application of this cutoff as a surrogate for estimating the biosolubility HMW polymers in the lysosomes of alveolar macrophages (*e.g.*, artificial lysosomal fluid).

The above screening criteria for respirability, reactivity, and biosolubility provide a framework for determining inclusion/exclusion from the HMW polymer category, as shown in Scheme 2. The screening criteria may be used for determining whether further evaluation of the new chemical substance is warranted using the tiered-testing strategy discussed later in this document.

Scheme [SEQ Scheme * ARABIC]. Framework for determining whether a chemical substance is included/excluded from the HMW polymer category.



Based on the above information, the HMW polymer category was defined to include a variety of respirable, non-reactive (*i.e.*, low toxicity), and poorly soluble HMW (*i.e.*, ≥ 10,000 Daltons)

materials, which meet the above-stated criteria for these parameters. HMW polymers meeting these criteria are those which are typically formed through various polymerization processes. Chemical substances included are branched and linear polymers, as well as co-polymers produced by random, block, graft, or other techniques. Crosslinked polymers were included in the category because crosslinking can decrease water solubility, but crosslinking was not necessary for inclusion. Therefore, the representative members of this category were refined to include polyacrylates/methacrylates, polyvinyl polymers, polyamides, and polyurethanes/polyureas. The water-dispersible forms polyacrylates/metacrylates and polyurethanes/polyureas would not present hazards for lung overload and are not included in the HMW polymer category [ADDIN EN.CITE ADDIN EN.CITE.DATA]; however, despite their exclusion from the category, they would need to be assessed for other potential hazard concerns. A summary of the structural features of these chemical substances and the chemical boundaries that were established is shown in [REF _Ref46674591 \h * MERGEFORMAT].

[EMBED ChemDraw.Document.6.0]

Figure [SEQ Figure * ARABIC]. Representative members of the HMW polymer category. Structure A is representative of polyacrylate/methacylate members, where R is H or methyl; R' and R'' are typically alkyl or substituted alkyl, although there are currently no limits on the substituents. However, charged groups such as carboxyl groups or amine groups would tend to make the polymer dispersible in water rather than insoluble in water. R' may be the same as R'' or different. This example represents a polymer containing one or two monomers, although sub-category members may comprise any number of monomers. Acrylamide and methacrylamide monomers (NR'_2 replaces OR' or OR") may also be present. Structure B is representative of polyvinyl members, where R is H or C1-C > 20. R' is typically methyl, CN, acetyloxy, Ph or Cl, although there are no current limits on R'. R' may be the same as R'' or different. This example represents a polymer containing one or two monomers, although sub-category members may comprise any number of monomers. Copolymers (e.g., including both acrylate/methacrylate and vinyl monomers) are also members of this category. Structure C is representative of the polyamides group and is made of condensation polymers in which the linkages are all amide functional groups. An example is polycaprolactam, shown.

Hazard Identification

TSCA and its implementing regulations do not require upfront testing on new chemical substances. Therefore, when assessing new chemical substances, EPA generally identifies toxicological analogues to inform the potential hazards for the new chemical substances. The

systematic review of the literature was used to identify inhalation studies that assessed endpoints indicative of "overload" for potential toxicological analogues. For the purpose of defining this chemical category, overload has the same definition as identified by EPA (1996) [ADDIN EN.CITE <EndNote><Cite><Author>EPA</Author><Year>1996</Year><RecNum>59</RecNum><DisplayText>[35]</DisplayText><record><rec-number>59</rec-number><foreign-keys><key app="EN" db-id="xs0a90va7aasfwex5aev0dvyp0t59sta5dae" timestamp="1595797014">59</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>Air Quality Criteria for Particulate Matter, Volume II of III</title><secondary-title>Office of Research and Development, U.S. Environmental Protection Agency, Washington, DC 20460</secondary-title></titles><periodical><full-title>Office of Research and Development, U.S. Environmental Protection Agency, Washington, DC 20460</full-title></periodical><pages>774, http://ofmpub.epa.gov/eims/eimscomm.getfile?p_download_id=219821</pages><volume>EPA/600/P-95/001bF</volume><dates><year>1996</year></dates><urls></urls></record></Cite></EndNote>]: "This is defined as the overwhelming of macrophage-mediated clearance by the deposition of particles at a rate which exceeds the capacity of that clearance pathway. It is a nonspecific effect noted in experimental studies, generally in rats, using many different kinds of poorly soluble particles (including TiO_2, volcanic ash, diesel exhaust particles, carbon black, and fly ash) and results in A [alveolar] region clearance slowing or stasis, with an associated inflammation and aggregation of macrophages in the lungs and increased translocation of

particles into the interstitium." The relevant studies that were identified are summarized below, followed by the selection of studies on toxicological analogues that may serve as representative points of departure for assessing the potential hazard for overload of some new chemical substances.

Human Data

The hazard concerns discussed herein are limited to chronic effects in the lower respiratory tract of rats exposed to HMW polymers. Epidemiological studies have shown increased lung burdens in workers chronically exposed to poorly soluble particles (PSPs), such as former coal miners; however, studies have shown that rodent models overpredict lung burdens in humans if adjustments are not made for kinetic differences in clearance and retention [ADDIN EN.CITE ADDIN EN.CITE.DATA]. This is consistent with findings from well-conducted epidemiological studies, which have not identified an association between occupational exposure to PSPs and an increased cancer risk. Oberdorster (1995) [ADDIN EN.CITE <EndNote><Cite><Author>Oberdorster</Author><Year>1995</Year><RecNum>60</RecNum><DisplayText>[36]</DisplayText><record><rec-number>60</rec-number><foreign-keys><key app="EN" db-id="xs0a90va7aasfwex5aev0dvyp0t59sta5dae" timestamp="1595797677">60</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Oberdorster, G.</author></authors></contributors><titles><title>Lung Particle Overload: Implications for Occupational Exposures to Particles</title><secondary-title>Regul Toxicol Pharmacol</secondary-title></titles><periodical><full-title>Regul Toxicol Pharmacol</full-title></periodical><pages>123-

135</pages><volume>27</volume><dates><year>1995</year></dates><urls></urls></record></Cite></EndNote>] concluded that "evidence in humans suggest that particle-overloaded lungs, *e.g.*, in coal workers, respond with fibrosis, but no increased incidence in lung tumors has been found in this group". It has also been reported that "epidemiological data from production workers demonstrate no correlation between PSP exposure and lung cancer or other non-malignant respiratory diseases" [ADDIN EN.CITE ADDIN EN.CITE.DATA]. Though these investigations focused on PSPs, the available, yet limited data on HMW polymers provide comparable results. For example, in a recent retrospective study of Xerox workers employed between 1960 and 1982, workers exposed to toner did not show an increased risk of "all-cause" or "cause-specific" mortality. The categories evaluated included cancer (*e.g.*, lung), diabetes, cardiovascular disease, and others [ADDIN EN.CITE ADDIN EN.CITE.DATA]. Aside from this one epidemiological study on toner exposures, the available studies that evaluated potential hazards from exposures to HMW polymers were limited to inhalation studies conducted in experimental animals as summarized below and described in further detail in Section 2 "Experimental Animal Inhalation Studies on HMW Polymers" of the Supplemental Information file.

Animal Data - Noncancer Effects

Inhalation studies performed in rats and hamsters have demonstrated effects ranging from inflammation to fibrosis after inhalation exposure to several HMW polymers including print toners comprised largely of styrene/butylmethacrylate copolymer and polyvinyl chloride dust. Several of these studies were conducted according to validated test guidelines and under good

laboratory practice (GLP) standards, and in some cases published in the peer-reviewed literature. A summary of these studies is provided below.

A series of sub-chronic and chronic studies were performed to test the inhalation effects of a water-insoluble styrene/butylmethacrylate polymer (the primary component of toner used in copy machines) of MW 70,000 in rats. In a subchronic 13-week study, rats were exposed to aerosol concentrations of toner at 0, 1, 4, 16, and 64 mg/m^3 (MMAD = 4 μm; GSD = 1.5; density = 1.15 g/cm^3) for 6 hours/day, 5 days/week. Dose-related increased lung weight and histological lesions (thickening of alveolar structure due to hypertrophy and hyperplasia of Type II cells) were seen in animals exposed to 16 and 64 mg/m^3. These exposure concentrations also resulted in a dose-related decrease in lung clearance, as measured by the retained quantity of the test substance in excised lungs, and increased lung particle burden [ADDIN EN.CITE <EndNote><Cite><Author>Muhle</Author><Year>1990</Year><RecNum>14</RecNum><DisplayText>[39]</DisplayText><record><rec-number>14</rec-number><foreign-keys><key app="EN" db-id="xs0a90va7aasfwex5aev0dvyp0t59sta5dae" timestamp="1590846288">14</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Muhle, H.</author><author>Bellmann, B.</author><author>Creutzenberg, O.</author><author>Fuhst, R.</author><author>Koch, W.</author><author>Mohr, U.</author><author>Takenaka, S.</author><author>Morrow, P.</author><author>Kilpper, R.</author><author>Mackenzie, J.</author><author>Mermelstein, R.</author></authors></contributors><titles><title>Subchronic Inhalation Study of Toner in Rats</title><secondary-title>Inhalation Toxicology</secondary-title></titles><periodical><full-

title>Inhalation Toxicology</full-title></periodical><pages>341-360</pages><volume>2</volume><number>4</number><dates><year>1990</year></dates><urls></urls><electronic-resource-num>https://doi.org/10.3109/08958379009145262</electronic-resource-num></record></Cite></EndNote>]. The NOAEC from this study was 4 mg/m^3.

Bellmann *et al.* (1992) [ADDIN EN.CITE <EndNote><Cite><Author>Bellmann</Author><Year>1992</Year><RecNum>4</RecNum><DisplayText>[40]</DisplayText><record><rec-number>4</rec-number><foreign-keys><key app="EN" db-id="xs0a90va7aasfwex5aev0dvyp0t59sta5dae" timestamp="1590844601">4</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Bellmann, B.</author><author>Muhle, H.</author><author>Creutzenberg, O.</author><author>Mermelstein, R.</author></authors></contributors><auth-address>Fraunhofer-Institut fur Toxikologie und Aerosolforschung, Hannover, Germany.</auth-address><titles><title>Irreversible pulmonary changes induced in rat lung by dust overload</title><secondary-title>Environ Health Perspect</secondary-title></titles><periodical><full-title>Environ Health Perspect</full-title></periodical><pages>189-91</pages><volume>97</volume><edition>1992/07/01</edition><keywords><keyword>Animals</keyword><keyword>Bronchoalveolar Lavage Fluid/*enzymology/*pathology</keyword><keyword>Cell Count</keyword><keyword>Dust</keyword><keyword>Environmental Exposure/*adverse effects</keyword><keyword>Female</keyword><keyword>Glucuronidase/metabolism</keyword><keyword>L-Lactate Dehydrogenase/metabolism</keyword><keyword>Lung/*drug

effects/enzymology</keyword><keyword>Macrophages/*drug effects/pathology/physiology</keyword><keyword>Phagocytosis/drug effects</keyword><keyword>Polymers/*adverse effects</keyword><keyword>Rats</keyword><keyword>Time Factors</keyword></keywords><dates><year>1992</year><pub-dates><date>Jul</date></pub-dates></dates><isbn>0091-6765 (Print)
0091-6765 (Linking)</isbn><accession-num>1396457</accession-num><urls><related-urls><url>https://www.ncbi.nlm.nih.gov/pubmed/1396457</url></related-urls></urls><custom2>PMC1519531</custom2><electronic-resource-num>10.1289/ehp.9297189</electronic-resource-num></record></Cite></EndNote>] performed an additional 13-week study using the same test substance used by Muhle *et al*. (1990) [ADDIN EN.CITE <EndNote><Cite><Author>Muhle</Author><Year>1990</Year><RecNum>14</RecNum><DisplayText>[39]</DisplayText><record><rec-number>14</rec-number><foreign-keys><key app="EN" db-id="xs0a90va7aasfwex5aev0dvyp0t59sta5dae" timestamp="1590846288">14</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Muhle, H.</author><author>Bellmann, B.</author><author>Creutzenberg, O.</author><author>Fuhst, R.</author><author>Koch, W.</author><author>Mohr, U.</author><author>Takenaka, S.</author><author>Morrow, P.</author><author>Kilpper, R.</author><author>Mackenzie, J.</author><author>Mermelstein, R.</author></authors></contributors><titles><title>Subchronic Inhalation Study of Toner in Rats</title><secondary-title>Inhalation Toxicology</secondary-title></titles><periodical><full-

title>Inhalation Toxicology</full-title></periodical><pages>341-360</pages><volume>2</volume><number>4</number><dates><year>1990</year></dates><urls></urls><electronic-resource-num>https://doi.org/10.3109/08958379009145262</electronic-resource-num></record></Cite></EndNote>] and included an extended 15-month post-exposure monitoring period. Rats were exposed to aerosol concentrations of toner at 0, 10, or 40 mg/m^3 (MMAD = 4 µm; GSD = 1.5; density = 1.15 g/cm^3) for 6 hours/day, 5 days/week. The study authors measured retention of the toner in the lungs and lung-associated lymph nodes (LALN) by photometric determination in dissolved tissues; clearance was monitored using tracer particles, and pulmonary effects were identified from enzymatic activities and differential cell counts in bronchoalveolar lavage fluid (BALF). The study authors identified clearance half-lives of 277 and 2,845 days for the low- and high-dose exposure groups, respectively, and reported pulmonary effects, as evidenced by increases in protein and enzyme markers of tissue damage in BALF that were partially reversible at 10 mg/m^3 and not reversible at 40 mg/m^3 [ADDIN EN.CITE <EndNote><Cite><Author>Bellmann</Author><Year>1992</Year><RecNum>4</RecNum><DisplayText>[40]</DisplayText><record><rec-number>4</rec-number><foreign-keys><key app="EN" db-id="xs0a90va7aasfwex5aev0dvyp0t59sta5dae" timestamp="1590844601">4</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Bellmann, B.</author><author>Muhle, H.</author><author>Creutzenberg, O.</author><author>Mermelstein, R.</author></authors></contributors><auth-address>Fraunhofer-Institut fur Toxikologie und Aerosolforschung, Hannover, Germany.</auth-address><titles><title>Irreversible pulmonary changes induced in rat lung by dust overload</title><secondary-title>Environ Health

Perspect</secondary-title></titles><periodical><full-title>Environ Health Perspect</full-title></periodical><pages>189-91</pages><volume>97</volume><edition>1992/07/01</edition><keywords><keyword>Animals</keyword><keyword>Bronchoalveolar Lavage Fluid/*enzymology/*pathology</keyword><keyword>Cell Count</keyword><keyword>Dust</keyword><keyword>Environmental Exposure/*adverse effects</keyword><keyword>Female</keyword><keyword>Glucuronidase/metabolism</keyword><keyword>L-Lactate Dehydrogenase/metabolism</keyword><keyword>Lung/*drug effects/enzymology</keyword><keyword>Macrophages/*drug effects/pathology/physiology</keyword><keyword>Phagocytosis/drug effects</keyword><keyword>Polymers/*adverse effects</keyword><keyword>Rats</keyword><keyword>Time Factors</keyword></keywords><dates><year>1992</year><pub-dates><date>Jul</date></pub-dates></dates><isbn>0091-6765 (Print)
0091-6765 (Linking)</isbn><accession-num>1396457</accession-num><urls><related-urls><url>https://www.ncbi.nlm.nih.gov/pubmed/1396457</url></related-urls></urls><custom2>PMC1519531</custom2><electronic-resource-num>10.1289/ehp.9297189</electronic-resource-num></record></Cite></EndNote>].

Muhle *et al.* (1991) [ADDIN EN.CITE <EndNote><Cite><Author>Muhle</Author><Year>1991</Year><RecNum>16</RecNum><DisplayText>[41]</DisplayText><record><rec-number>16</rec-number><foreign-keys><key app="EN" db-id="xs0a90va7aasfwex5aev0dvyp0t59sta5dae"

timestamp="1590846537">16</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Muhle, H.</author><author>Bellmann, B.</author><author>Creutzenberg, O.</author><author>Dasenbrock, C.</author><author>Ernst, H.</author><author>Kilpper, R.</author><author>Mackenzie, J. C.</author><author>Morrow, P.</author><author>Mohr, U.</author><author>Takenaka, S.</author><author>Mermelstein, R.</author></authors></contributors><auth-address>Xerox Corp,Joseph C Wilson Ctr Technol,Corp Environm Hlth,Webster,Ny 14580
Univ Rochester,Rochester,Ny 14642</auth-address><titles><title>Pulmonary Response to Toner Upon Chronic Inhalation Exposure in Rats</title><secondary-title>Fundamental and Applied Toxicology</secondary-title><alt-title>Fund Appl Toxicol</alt-title></titles><periodical><full-title>Fundamental and Applied Toxicology</full-title><abbr-1>Fund Appl Toxicol</abbr-1></periodical><alt-periodical><full-title>Fundamental and Applied Toxicology</full-title><abbr-1>Fund Appl Toxicol</abbr-1></alt-periodical><pages>280-299</pages><volume>17</volume><number>2</number><keywords><keyword>bronchoalveolar lavage fluid</keyword><keyword>diesel exhaust</keyword><keyword>toxicity</keyword><keyword>clearance</keyword></keywords><dates><year>1991</year><pub-dates><date>Aug</date></pub-dates></dates><isbn>0272-0590</isbn><accession-num>WOS:A1991FZ99700006</accession-num><urls><related-urls><url><Go to ISI>://WOS:A1991FZ99700006</url></related-urls></urls><electronic-resource-num>Doi 10.1016/0272-0590(91)90219-T</electronic-resource-num><language>English</language></record></Cite></EndNote>] and Bellmann *et al.* (1991) [ADDIN EN.CITE <EndNote><Cite><Author>Bellmann</Author><Year>1991</Year><RecNum>3</RecNum><

DisplayText>[42]</DisplayText><record><rec-number>3</rec-number><foreign-keys><key app="EN" db-id="xs0a90va7aasfwex5aev0dvyp0t59sta5dae" timestamp="1590844572">3</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Bellmann, B.</author><author>Muhle, H.</author><author>Creutzenberg, O.</author><author>Dasenbrock, C.</author><author>Kilpper, R.</author><author>MacKenzie, J. C.</author><author>Morrow, P.</author><author>Mermelstein, R.</author></authors></contributors><auth-address>Fraunhofer-Institut fur Toxikologie und Aerosolforschung, Hannover, Germany.</auth-address><titles><title>Lung clearance and retention of toner, utilizing a tracer technique, during chronic inhalation exposure in rats</title><secondary-title>Fundam Appl Toxicol</secondary-title></titles><periodical><full-title>Fundam Appl Toxicol</full-title></periodical><pages>300-13</pages><volume>17</volume><number>2</number><edition>1991/08/01</edition><keywords><keyword>Aerosols</keyword><keyword>Animals</keyword><keyword>Body Burden</keyword><keyword>Dust/adverse effects</keyword><keyword>Female</keyword><keyword>Half-Life</keyword><keyword>Lung/*metabolism</keyword><keyword>Lymph Nodes/metabolism</keyword><keyword>Male</keyword><keyword>Pulmonary Alveoli/metabolism</keyword><keyword>Rats</keyword><keyword>Rats, Inbred F344</keyword><keyword>Sex Factors</keyword><keyword>Silicon Dioxide/*metabolism</keyword><keyword>Titanium/*metabolism</keyword></keywords><dates><year>1991</year><pub-dates><date>Aug</date></pub-dates></dates><isbn>0272-0590 (Print)
0272-0590 (Linking)</isbn><accession-num>1662649</accession-

num><urls><related-urls><url>https://www.ncbi.nlm.nih.gov/pubmed/1662649</url></related-urls></urls><electronic-resource-num>10.1016/0272-0590(91)90220-x</electronic-resource-num></record></Cite></EndNote>] reported findings from a chronic 24-month exposure study in rats exposed to toner at aerosol concentrations of 0, 1, 4, or 16 mg/m^3 (MMAD = 4 µm; GSD = 1.5; density = 1.15 g/cm^3) for 6 hours/day, 5 days/week. The study was performed according to OECD No. 453 Combined Chronic Toxicity/Carcinogenicity Studies and under GLP standards. The study authors reported dose-related impaired particle clearance, elevated lung particle burden, and lung effects (fibrosis, BALF markers of tissue damage, and increased lung weight) at 4 and 16 mg/m^3, with a NOAEC of 1 mg/m^3.

Unpublished subchronic (3 months) and chronic (18 months) hamster studies of the same print toner tested by Muhle *et al.* (1990, 1991) and Bellman *et al.* (1991, 1992) [ADDIN EN.CITE ADDIN EN.CITE.DATA] showed effects similar to those in rats [ADDIN EN.CITE ADDIN EN.CITE.DATA]. The unpublished 3-month study was hampered by disease and mortality unrelated to treatment. In the unpublished 18-month study, the hamsters were exposed to concentrations of 0, 1.5, 6, or 24 mg/m^3 for the first 5 months and then concentrations of 0, 4, 16, or 64 mg/m^3 for the remaining time. At all exposure concentrations, the hamsters exhibited macrophage accumulation, interstitial inflammatory cell infiltration, and bronchiolar/alveolar hyperplasia, along with particle deposits and lymphatic hyperplasia in the LALNs. At the mid- and high-exposure concentrations, fibrosis and alveolar PMN infiltration were noted at the end of exposure and/or after the 5 month post-exposure recovery period; the highest exposure group also exhibited increased lung weight and effects on BALF parameters (increased cell number,

macrophage count, LDH, β glucuronidase, total protein, and hydroxyproline). The LOAEC for this study was in the range of 1.5 to 4 mg/m^3.

Muhle *et al.* (1990) [ADDIN EN.CITE <EndNote><Cite><Author>Muhle</Author><Year>1990</Year><RecNum>13</RecNum><DisplayText>[46]</DisplayText><record><rec-number>13</rec-number><foreign-keys><key app="EN" db-id="xs0a90va7aasfwex5aev0dvyp0t59sta5dae" timestamp="1590845894">13</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Muhle, H.</author><author>Bellmann, B.</author><author>Creutzenberg, O.</author><author>Heinrich, U.</author><author>Ketkar, M.</author><author>Mermelstein, R.</author></authors></contributors><titles><title>Dust overloading of lungs after exposure of rats to particles of low solubility: Comparative studies</title><secondary-title>Journal of Aerosol Science</secondary-title></titles><periodical><full-title>Journal of Aerosol Science</full-title></periodical><pages>374-377</pages><volume>21</volume><number>3</number><dates><year>1990</year></dates><urls></urls><electronic-resource-num>https://doi.org/10.1016/0021-8502(90)90062-3</electronic-resource-num></record></Cite></EndNote>] performed an eight-month inhalation study in rats exposed to an aerosol of PVC powder at 0, 3.3, 8.3, or 20.2 mg/m^3 (MMAD = 1.3 µm; GSD = 2.07; density = 1.3 g/cm^3) for 5 hours/day, 5 days/week. Retention, clearance, and pulmonary effects were evaluated, as reported previously by these same authors. Using radiolabeled (^{85}Sr) polystyrene particles as tracers, these authors showed that pulmonary clearance was significantly decreased in rats after seven months of exposure (25 hours per week)

to PVC powder at concentrations ≥ 3.3 mg/m^3. Mean alveolar clearance half-times increased with exposure from 1.2-fold higher than controls to 3.2-fold higher than controls at concentrations from 3.3 to 20.2 mg/m^3. The study authors calculated half-times for alveolar clearances of 71, 122, and 184 days at exposure concentrations of 3.3, 8.3, and 20.2 mg/m^3, supporting that lung overload occurred at concentrations ≥ 3.3 mg/m^3 for this water-insoluble polymer.

Animal Data - Cancer

Chronic inhalation exposure data specifically pertaining to HMW polymers are limited to a 24-month rat study of print toner and an 18-month hamster study of print toner [ADDIN EN.CITE <EndNote><Cite><Author>Muhle</Author><Year>1991</Year><RecNum>16</RecNum><DisplayText>[41]</DisplayText><record><rec-number>16</rec-number><foreign-keys><key app="EN" db-id="xs0a90va7aasfwex5aev0dvyp0t59sta5dae" timestamp="1590846537">16</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Muhle, H.</author><author>Bellmann, B.</author><author>Creutzenberg, O.</author><author>Dasenbrock, C.</author><author>Ernst, H.</author><author>Kilpper, R.</author><author>Mackenzie, J. C.</author><author>Morrow, P.</author><author>Mohr, U.</author><author>Takenaka, S.</author><author>Mermelstein, R.</author></authors></contributors><auth-address>Xerox Corp,Joseph C Wilson Ctr Technol,Corp Environm Hlth,Webster,Ny 14580
Univ Rochester,Rochester,Ny 14642</auth-address><titles><title>Pulmonary Response to Toner Upon Chronic Inhalation Exposure in Rats</title><secondary-title>Fundamental and Applied Toxicology</secondary-title><alt-title>Fund Appl Toxicol</alt-title></titles><periodical><full-

title>Fundamental and Applied Toxicology</full-title><abbr-1>Fund Appl Toxicol</abbr-1></periodical><alt-periodical><full-title>Fundamental and Applied Toxicology</full-title><abbr-1>Fund Appl Toxicol</abbr-1></alt-periodical><pages>280-299</pages><volume>17</volume><number>2</number><keywords><keyword>bronchoalveolar lavage fluid</keyword><keyword>diesel exhaust</keyword><keyword>toxicity</keyword><keyword>clearance</keyword></keywords><dates><year>1991</year><pub-dates><date>Aug</date></pub-dates></dates><isbn>0272-0590</isbn><accession-num>WOS:A1991FZ99700006</accession-num><urls><related-urls><url><Go to ISI>://WOS:A1991FZ99700006</url></related-urls></urls><electronic-resource-num>Doi 10.1016/0272-0590(91)90219-T</electronic-resource-num><language>English</language></record></Cite></EndNote>]. No increased tumor incidence was observed in either study; however, interstitial and alveolar lung pathology has been documented in long-term inhalation studies on these polymers. See section on "Animal Data - Noncancer Effects" above.

Supporting Information

An *in vitro* study was identified and reviewed that may be relevant for determining the reactivity/non-reactivity of HMW polymers that do not meet the initial FG and/or FGEW screening criteria.

Wiemann et al. (2016) [ADDIN EN.CITE ADDIN EN.CITE.DATA] developed an *in vitro* assay to test nanoparticles for biologically active toxicity from passive (*i.e.*, overload condition) toxicity. The assay uses rat NR8383 alveolar macrophages incubated with test material in cell

culture medium, and assesses toxicity *via* measurement of LDH, glucuronidase, and tumor necrosis factor α (TNFα) (after 16 hours exposure), and hydrogen peroxide (after 1.5 hours) in the cell culture supernatant. The authors tested 18 inorganic nanomaterials using the assay, as well as corundum as a negative control and quartz DQ12 as a positive control. Based on data from short term inhalation studies, each test material was categorized as either active (NOAEC <10 mg/m^3 for adverse inflammatory action in a 5-day inhalation study) or passive (*i.e.*, inducing nonspecific cell overload). The *in vitro* assay threshold for active toxicity was a surface-area/volume concentration of 6,000 mm^2/mL (calculated as particle or agglomerate Brunauer Teller and Emmett [BET] surface area × mass concentration in μg/mL) in at least two of the four parameters measured in supernatant. The nanomaterials tested showed good correspondence between the *in vitro* and *in vivo* parameters (assay accuracy 95%), suggesting that, the assay could be useful in distinguishing specific ("active") toxicity from nonspecific ("passive" or overload) effects on alveolar macrophages. Although only nanoparticles were tested by these authors, this assay may be useful for screening out HMW polymers for inclusion/exclusion in the category, *e.g.*, those identified as "active" would be inconsistent with the low-concern level and inclusion in the category, whereas those identified as "passive" appear to be consistent with inclusion. Additionally, this assay could be useful for screening polymers with specific toxicities (*i.e.*, excluded from overload category) prior to *in vivo* testing of "overload" for passive polymers.

Quantitative Points of Departure (PODs)

A single epidemiological study of inhaled HMW polymers was identified - the retrospective study of Xerox workers [ADDIN EN.CITE ADDIN EN.CITE.DATA]. This study did not

report exposure concentrations associated with the evaluated health outcomes and is therefore not useful for determining quantitative PODs for pulmonary effects of HMW polymers.

A summary of animal studies documenting pulmonary effects after exposure to HMW polymers and the PODs identified from them is provided in [REF _Ref46678612 \h * MERGEFORMAT]. The PODs presented in the table include those from studies meeting the following criteria:

- Exposure was *in vivo via* inhalation (*in vitro*, intratracheal instillation studies were not included);
- Exposure continued for at least 13 weeks; and
- Critical study information was reported, including exposure concentrations, exposure frequency, and aerodynamic particle size (MMAD and GSD).

Each study was evaluated to determine whether the data were amenable for BMD modeling.

For the polyacrylates and methacrylates subcategory, several subchronic studies are included in [REF _Ref46678612 \h * MERGEFORMAT] that met the initial POD selection criteria; however, BMD modeling was not performed on these studies because chronic studies were available and deemed more relevant for the hazard assessment. Two chronic studies met the POD selection criteria: the published 24-month rat study of 9000 type toner and the unpublished 18-month hamster study of the same toner [ADDIN EN.CITE ADDIN EN.CITE.DATA]. BMD modeling was performed for the data in the rat study performed by Muhle *et al.* (1991) [ADDIN EN.CITE

<EndNote><Cite><Author>Muhle</Author><Year>1991</Year><RecNum>16</RecNum><Di
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type><contributors><authors><author>Muhle, H.</author><author>Bellmann,
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C.</author><author>Morrow, P.</author><author>Mohr, U.</author><author>Takenaka,
S.</author><author>Mermelstein, R.</author></authors></contributors><auth-address>Xerox
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Univ
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299</pages><volume>17</volume><number>2</number><keywords><keyword>bronchoalveo
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resource-num>Doi 10.1016/0272-0590(91)90219-T</electronic-resource-

num><language>English</language></record></Cite></EndNote>], as it used a longer exposure duration, was published in a peer-reviewed journal, and did not change exposure concentrations during the study, whereas, in the hamster study, exposure concentrations were modified after the first five months. Among the endpoints affected at the LOAEC in that study (macrophages, PMN, and lymphocytes in BAL; incidence of pulmonary fibrosis), only the fibrosis incidence could be modeled, as the BALF parameters were reported without measures of variability (*i.e.*, standard deviation or standard error). The incidences of lung fibrosis (summed across severity categories) were subjected to BMD modeling, as described in Section 3 "Benchmark Dose (BMD) Modeling Outputs" of the Supplemental Information file. The BMCL from the best-fitting model was 2.5 mg/m^3, as shown in [REF _Ref46678612 \h * MERGEFORMAT].

Only a single study was available for the polyvinyl subcategory; however, BMD modeling on the alveolar clearance for the tracer was not possible because of the absence of reported measures of variability ([REF _Ref46678612 \h * MERGEFORMAT]).

Table [SEQ Table * ARABIC]. Available PODs for inhalation studies on HMW Polymers.

Test material	Strain, Species, Sex, Exposure frequency and duration, Recovery	Exposure Concentrations (mg/m^3)	NOAEC (mg/m^3)	LOAEC (mg/m^3)	BMCL (mg/m^3)	Lung Effects at LOAEC	Reference
Polyacrylates and Methacrylates Sub-category							
9000 Toner (styrene/butylmethacrylate random copolymer)	SPF F344 rats, male and female (288/group); 24 months (6 hr/d, 5 d/wk), 2 months recovery	0, 1, 4, or 16	1	4	2.5 (fibrosis)	Significantly decreased macrophages and increased PMN and lymphocytes in BAL; significantly increased incidence of minimal to mild pulmonary fibrosis	[ADDIN EN.CITE ADDIN EN.CITE.DATA]

9000 Toner (styrene/butylmethacrylate random copolymer)	Syrian Golden Han:AURA Hamster, male and female, (50/group); 18 months (6 hr/d, 5 d/wk); 3-5 mo. recovery	0, 1.5, 6, or 24 (months 1-5); 0, 4, 16, or 64 (months 6-18)	ND	1.5-4	Not derived; variable exposure regimen	Significantly increased incidences of bronchiolar/alveolar hyperplasia (males); accumulation particle-laden macrophages in lungs; interstitial inflammatory cell infiltration in lungs (males); lymphatic hyperplasia in LALN (males); and particle deposits in LALN	[ADDIN EN.CITE <EndNote><Cite><Author>Institute</Author><Year>1991</Year><RecNum>30</RecNum><DisplayText>[49]</DisplayText><record><rec-number>30</rec-number><foreign-keys><key app="EN" db-id="xs0a90va7aasfwex5aev0dvyp0t59sta5dae" timestamp="1590849152">30</key></foreign-keys><ref-type name="Unpublished Work">34</ref-type><contributors><authors><author>Fraunho

							fer Institute</author></authors></contributors><titles><title>An investigation of the biological effects of a toner fraction. Final Report of a subchronic inhalation study in Syrian Golden hamsters. Submitted to the U.S. Environmental Protection Agency under TSCA Section 8E. OTS0513473-2</title></titles><dates><year>1991</year></dates><publisher>Xerox Corporation</publisher

Table [SEQ Table * ARABIC]. Available PODs for inhalation studies on HMW Polymers.

Test material	Strain, Species, Sex, Exposure frequency and duration, Recovery	Exposure Concentrations (mg/m^3)	NOAEC (mg/m^3)	LOAEC (mg/m^3)	BMCL (mg/m^3)	Lung Effects at LOAEC	Reference
							><urls></urls></record></Cite></EndNote>]

Toner A (styrene/butylmethacrylate random copolymer)	F344/CrlBR rat, female, (58-66/group); 3 months (6 hr/d, 5 d/wk); up to 6 mo. recovery	0, 4, 16, or 64	ND	4	Not derived	Significantly increased incidence slight to moderate accumulation of particle-laden macrophages in lungs	[ADDIN EN.CITE <EndNote><Cite><Author>Institute</Author><Year>1991</Year><RecNum>28</RecNum><DisplayText>[43]</DisplayText><record><rec-number>28</rec-number><foreign-keys><key app="EN" db-id="xs0a90va7aasfwex5aev0dvyp0t59sta5dae" timestamp="1590848985">28</key></foreign-keys><ref-type name="Unpublished Work">34</ref-type><contributors><authors><author>Fraunho

							fer Institute</author></authors></contributors><titles><title>An investigation of the biological effects of a toner fraction in a subchronic inhalation study in rats (Toner A). Final Report. Submitted to the U.S. Environmental Protection Agency under TSCA Section 8E. OTS0513473-7</title></titles><dates><year>1991</year></dates><publisher>Xerox Corporation</publisher><urls></urls></record

Table [SEQ Table * ARABIC]. Available PODs for inhalation studies on HMW Polymers.

Test material	Strain, Species, Sex, Exposure frequency and duration, Recovery	Exposure Concentrations (mg/m^3)	NOAEC (mg/m^3)	LOAEC (mg/m^3)	BMCL (mg/m^3)	Lung Effects at LOAEC	Reference
							></Cite></ EndNote>]

9000 Toner (styrene/butylmethacrylate random copolymer)	SPF F344 rat, female (≥18/group); 3 months (6 hr/d, 5 d/wk), 15 months recovery	0, 10, or 40	ND	10	Not derived	Significantly decreased alveolar clearance	[ADDIN EN.CITE <EndNote><Cite><Author>Bellmann</Author><Year>1992</Year><RecNum>4</RecNum><DisplayText>[40]</DisplayText><record><rec-number>4</rec-number><foreign-keys><key app="EN" db-id="xs0a90va7aasfwex5aev0dvyp0t59sta5dae" timestamp="1590844601">4</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Bellman

							n, B.</author><author>Muhle, H.</author><author>Creutzenberg, O.</author><author>Mermelstein, R.</author></authors></contributors><auth-address>Fraunhofer-Institut fur Toxikologie und Aerosolforschung, Hannover, Germany.</auth-address><titles><title>Irreversible pulmonary changes induced in rat lung by dust overload</title><secondary-title>Environ Health Perspect</secondary-title></titles

							><periodical><full-title>Environ Health Perspect</full-title></periodical><pages>189-91</pages><volume>97</volume><edition>1992/07/01</edition><keywords><keyword>Animals</keyword><keyword>Bronchoalveolar Lavage Fluid/*enzymology/*pathology</keyword><keyword>Cell Count</keyword><keyword>Dust</keyword><keyword>Environmental Exposure/*adverse effects</keyword><keyword>Female</keywor

							d><keyword>Glucuronidase/metabolism</keyword><keyword>L-Lactate Dehydrogenase/metabolism</keyword><keyword>Lung/*drug effects/enzymology</keyword><keyword>Macrophages/*drug effects/pathology/physiology</keyword><keyword>Phagocytosis/drug effects</keyword><keyword>Polymers/*adverse effects</keyword><keyword>Rats</keyword><keyword>Time Factors</keyword></keywords><d

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0091-6765 (Linking)</isbn><accession-num>1396457</accession-num><urls><related-urls><url>https://www.ncbi.nlm.nih.gov/pubmed/1396457</url></related-urls></urls><custom2>PMC1519531</custom2><electronic-resource-num>10.1289/ehp.9297189</electronic-resource-num></record></Cite>

Table [SEQ Table * ARABIC]. Available PODs for inhalation studies on HMW Polymers.

Test material	Strain, Species, Sex, Exposure frequency and duration, Recovery	Exposure Concentrations (mg/m^3)	NOAEC (mg/m^3)	LOAEC (mg/m^3)	BMCL (mg/m^3)	Lung Effects at LOAEC	Reference
							</EndNote>]

9000 Toner (styrene/butylmethacrylate random copolymer)	SPF F344 rat, male and female (56-74/sex/group); 3 months (6 hr/d, 5 d/wk), 3 months recovery	0, 1, 4, 16, or 64	4	16	Not derived	Significantly increased relative lung weight in males; histopathology showed a few particles in alveolar walls and a slight degree of thickening of the alveolar structure due to hypertrophy and hyperplasia of Type II cells and accumulation of a few interstitial cells; slightly enlarged LALN; decreased alveolar clearance	[ADDIN EN.CITE <EndNote><Cite><Author>Muhle</Author><Year>1990</Year><RecNum>14</RecNum><DisplayText>[39]</DisplayText><record><rec-number>14</rec-number><foreign-keys><key app="EN" db-id="xs0a90va7aasfwex5aev0dvyp0t59sta5dae" timestamp="1590846288">14</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Muhle,

							H.</author><author>Bellmann, B.</author><author>Creutzenberg, O.</author><author>Fuhst, R.</author><author>Koch, W.</author><author>Mohr, U.</author><author>Takenaka, S.</author><author>Morrow, P.</author><author>Kilpper, R.</author><author>Mackenzie, J.</author><author>Mermelstein, R.</author></authors></contributors><titles><title>Subchronic Inhalation Study of Toner in Rats</title>

							<secondary-title>Inhalation Toxicology </secondary-title></titles><periodical><full-title>Inhalation Toxicology </full-title></periodical><pages>341-360</pages><volume>2</volume><number>4</number><dates><year>1990</year></dates><urls></urls><electronic-resource-num>https://doi.org/10.3109/08958379009145262</electronic-resource-num></record></Cite></EndNote>]

Toner B (styrene/ butadiene random copolymer)	F344 rat, female (50 rats/group for main study) up to 6 mo. recovery	0, 1, 4, 16, or 64	4	16	Not derived	Significantly increased incidence very slight to slight focal/multifocal interstitial inflammatory cell infiltration in lungs	[ADDIN EN.CITE <EndNote><Cite><Author>Institute</Author><Year>1991</Year><RecNum>29</RecNum><DisplayText>[50]</DisplayText><record><rec-number>29</rec-number><foreign-keys><key app="EN" db-id="xs0a90va7aasfwex5aev0dvyp0t59sta5dae" timestamp="1590849070">29</key></foreign-keys><ref-type name="Unpublished Work">34</ref-type><contributors><authors><author>Fraunho

							fer Institute</author></authors></contributors><titles><title>An investigation of the biological effects of a toner fraction in a subchronic inhalation study in rats (Toner B). Final Report. Submitted to the U.S. Environmental Protection Agency under TSCA Section 8E. OTS0513473-7</title></titles><dates><year>1991</year></dates><publisher>Xerox Corporation</publisher><urls></urls></record

Table [SEQ Table * ARABIC]. Available PODs for inhalation studies on HMW Polymers.

Test material	Strain, Species, Sex, Exposure frequency and duration, Recovery	Exposure Concentrations (mg/m^3)	NOAEC (mg/m^3)	LOAEC (mg/m^3)	BMCL (mg/m^3)	Lung Effects at LOAEC	Reference
							></Cite></ EndNote>]
Polyvinyls Sub-Category							

Polyvinyl chloride Powder	Rat, female (strain not reported); group sizes not reported; 8 months (25 hr/wk); up to 100 d recovery	0, 3.3, 8.3 or 20.2	ND	3.3	Not derived; missing SD/SE	Significantly decreased alveolar clearance	[ADDIN EN.CITE <EndNote><Cite><Author>Muhle</Author><Year>1990</Year><RecNum>13</RecNum><DisplayText>[46]</DisplayText><record><rec-number>13</rec-number><foreign-keys><key app="EN" db-id="xs0a90va7aasfwex5aev0dvyp0t59sta5dae" timestamp="1590845894">13</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Muhle,

							H.</author><author>Bellmann, B.</author><author>Creutzenberg, O.</author><author>Heinrich, U.</author><author>Ketkar, M.</author><author>Mermelstein, R.</author></authors></contributors><titles><title>Dust overloading of lungs after exposure of rats to particles of low solubility: Comparative studies</title><secondary-title>Journal of Aerosol Science</secondary-title></titles><periodica

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Table [SEQ Table * ARABIC]. Available PODs for inhalation studies on HMW Polymers.

Test material	Strain, Species, Sex, Exposure frequency and duration, Recovery	Exposure Concentrations (mg/m^3)	NOAEC (mg/m^3)	LOAEC (mg/m^3)	BMCL (mg/m^3)	Lung Effects at LOAEC	Reference
							l><full-title>Journal of Aerosol Science</full-title></periodical><pages>374-377</pages><volume>21</volume><number>3</number><dates><year>1990</year></dates><urls></urls><electronic-resource-num>https://doi.org/10.1016/0021-8502(90)90062-3</electronic-resource-num></record></Cite></EndNote>]

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Study Selection for establishing sub-category points of departure (PODs)

In rats, the key events in the development of lung tumors in rats in response to inhalation of inorganic PSPs (as outlined by ECETOC 2013 [ADDIN EN.CITE <EndNote><Cite><Author>ECETOC</Author><Year>2013</Year><RecNum>9</RecNum><DisplayText>[29]</DisplayText><record><rec-number>9</rec-number><foreign-keys><key app="EN" db-id="xs0a90va7aasfwex5aev0dvyp0t59sta5dae" timestamp="1590845309">9</key></foreign-keys><ref-type name="Report">27</ref-type><contributors><authors><author>ECETOC</author></authors></contributors><titles><title>Poorly Soluble Particles / Lung Overload</title></titles><pages>130, http://www.ecetoc.org/wp-content/uploads/2014/08/ECETOC-TR-122-Poorly-Soluble-Particles-Lung-Overload.pdf</pages><number>Technical Report No. 122</number><dates><year>2013</year><pub-dates><date>December 2013</date></pub-dates></dates><pub-location>Brussels, Belguim</pub-location><publisher>European Centre for Ecotoxicology and Toxicology of Chemicals</publisher><work-type>Technical Report</work-type><urls><related-urls><url>http://www.ecetoc.org/wp-content/uploads/2014/08/ECETOC-TR-122-Poorly-Soluble-Particles-Lung-Overload.pdf</url></related-urls></urls></record></Cite></EndNote>], Bevan *et al.*, 2018 [ADDIN EN.CITE ADDIN EN.CITE.DATA], Driscoll and Borm, 2020 [ADDIN EN.CITE <EndNote><Cite><Author>Driscoll</Author><Year>2020</Year><RecNum>40</RecNum><DisplayText>[52]</DisplayText><record><rec-number>40</rec-number><foreign-keys><key app="EN" db-id="xs0a90va7aasfwex5aev0dvyp0t59sta5dae" timestamp="1595775199">40</key></foreign-keys><ref-type name="Journal Article">17</ref-

type><contributors><authors><author>Driscoll, K. E.</author><author>Borm, P. J. A.</author></authors></contributors><auth-address>Healthcare Innovation Partners, Princeton, NJ, USA.
Ernest Mario School of Pharmacy, Rutgers University, Piscataway, NJ, USA.
Nanoconsult BV, Meerssen, The Netherlands.
Dusseldorf University, Dusseldorf, Germany.</auth-address><titles><title>Expert workshop on the hazards and risks of poorly soluble low toxicity particles</title><secondary-title>Inhal Toxicol</secondary-title><alt-title>Inhalation toxicology</alt-title></titles><alt-periodical><full-title>Inhalation Toxicology</full-title></alt-periodical><pages>53-62</pages><volume>32</volume><number>2</number><edition>2020/03/10</edition><keywords><keyword>*pslt</keyword><keyword>*hazard</keyword><keyword>*inhalation</keyword><keyword>*lung cancer</keyword><keyword>*lung particle overload</keyword><keyword>*particles</keyword><keyword>*risk</keyword></keywords><dates><year>2020</year><pub-dates><date>Feb</date></pub-dates></dates><isbn>0895-8378</isbn><accession-num>32149535</accession-num><urls></urls><electronic-resource-num>10.1080/08958378.2020.1735581</electronic-resource-num><remote-database-provider>NLM</remote-database-provider><language>eng</language></record></Cite></EndNote>]) are: (1) impaired pulmonary clearance, (2) persistent neutrophilic inflammation, (3) increased production of reactive oxygen species (ROS) and reactive nitrogen species (RNS), and (4) proliferation of cells initiated by secondary genotoxicity (from ROS, RNS, and/or inflammation) and tumor formation.

Though the key events for lung overload from HMW polymers have not been thoroughly studied, the available data as reviewed herein suggests that HMW polymers may lead to lung overload in the rat through similar key events. It should be noted that cytotoxicity to macrophages by a poorly soluble HMW polymer or components present in the polymer may negatively impact clearance *via* alveolar macrophages, thereby leading to tumor formation in humans. However, substances with these properties (*i.e.*, cytotoxicity) would not be included within the boundaries for the HMW polymers category.

Of the studies listed in [REF _Ref46678612 \h * MERGEFORMAT], PODs of 2.5 mg/m^3 and 3.3 mg/m^3 were identified for the polyacrylates/ methacrylates sub-category and the polyvinyls sub-category, respectively. The 24-month study on the 9000 Toner with a $BMCL_{10}$ of 2.5 mg/m^3 for pulmonary fibrosis was selected as a principle study for polyacrylates/methacrylates because it was the longest duration study on this sub-category of materials and was conducted in the most susceptible species for lung overload (*i.e.*, the rat). Muhle et al. (1990) [ADDIN EN.CITE <EndNote><Cite><Author>Muhle</Author><Year>1990</Year><RecNum>13</RecNum><DisplayText>[46]</DisplayText><record><rec-number>13</rec-number><foreign-keys><key app="EN" db-id="xs0a90va7aasfwex5aev0dvyp0t59sta5dae" timestamp="1590845894">13</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Muhle, H.</author><author>Bellmann, B.</author><author>Creutzenberg, O.</author><author>Heinrich, U.</author><author>Ketkar, M.</author><author>Mermelstein, R.</author></authors></contributors><titles><title>Dust overloading of lungs after exposure of rats to particles of low solubility: Comparative studies</title><secondary-title>Journal of Aerosol Science</secondary-

title></titles><periodical><full-title>Journal of Aerosol Science</full-title></periodical><pages>374-377</pages><volume>21</volume><number>3</number><dates><year>1990</year></dates><urls></urls><electronic-resource-num>https://doi.org/10.1016/0021-8502(90)90062-3</electronic-resource-num></record></Cite></EndNote>] was selected as a principle study for identifying a LOAEC of 3.3 mg/m^3 for the polyvinyls sub-category because it was based on decreased alveolar clearance, which is the first key event in the proposed adverse outcome pathway for lung overload from PSPs in the rat [ADDIN EN.CITE ADDIN EN.CITE.DATA]. These study PODs represent potential starting points for evaluating new chemical substances that fit within one of the HMW polymer sub-categories. EPA may determine that either of these PODs is an acceptable toxicological analogue for chemistries that do not fit within the sub-categories but are anticipated to have comparable or greater potential for causing lung overload in the rat than the new chemical substance under evaluation. For example, EPA generally uses the POD of 3.3 mg/m^3 for quantifying the potential risks of HMW polymers, even for chemistries that would not fall within the polyvinyls sub-category, based on the properties of the new chemical substance compared to the PVC powder. Notwithstanding this, we recognize that data on a new chemical substance or an alternative analogue would take precedence over using one of these analogues as the default POD, if EPA concludes there are no study limitations on the new chemical substance or alternative analogue that would preclude the use of those data.

Due to the limited data on HMW polymers, available knowledge about inorganic PSPs was used to make inferences about HMW polymers. Compared to systemic effects, lung overload responses to inorganic PSPs show large variations in susceptibility between and among

mammalian species, with the rat being the only species to develop lung tumors [ADDIN EN.CITE <EndNote><Cite><Author>ECETOC</Author><Year>2013</Year><RecNum>9</RecNum><DisplayText>[29]</DisplayText><record><rec-number>9</rec-number><foreign-keys><key app="EN" db-id="xs0a90va7aasfwex5aev0dvyp0t59sta5dae" timestamp="1590845309">9</key></foreign-keys><ref-type name="Report">27</ref-type><contributors><authors><author>ECETOC</author></authors></contributors><titles><title>Poorly Soluble Particles / Lung Overload</title></titles><pages>130, http://www.ecetoc.org/wp-content/uploads/2014/08/ECETOC-TR-122-Poorly-Soluble-Particles-Lung-Overload.pdf</pages><number>Technical Report No. 122</number><dates><year>2013</year><pub-dates><date>December 2013</date></pub-dates></dates><pub-location>Brussels, Belguim</pub-location><publisher>European Centre for Ecotoxicology and Toxicology of Chemicals</publisher><work-type>Technical Report</work-type><urls><related-urls><url>http://www.ecetoc.org/wp-content/uploads/2014/08/ECETOC-TR-122-Poorly-Soluble-Particles-Lung-Overload.pdf</url></related-urls></urls></record></Cite></EndNote>]. This species-specific response has been explained by species differences in the accumulation of insoluble and respirable particles in the lungs, although cytotoxicity is also an issue with some inorganic PSPs (*e.g.*, crystalline silica). For example, humans are at least six times more resistant to attaining lung overload conditions than rats for the following reasons: human alveolar macrophages (AMs) are larger (*i.e.*, average volume = 4,990 μm^3) than rat AMs (*i.e.*, average volume = 1,166 μm^3); humans have a greater number of AMs (*i.e.*, average = 7.0×10^9) than rats (*i.e.*, average = 2.6×10^7); and human AMs patrol a smaller surface area (*i.e.*, average = 22,000 μm^2/AM) than

rat AMs (*i.e.*, average = 140,000 μm^2/AM) [ADDIN EN.CITE ADDIN EN.CITE.DATA]. Further, the site of retention for poorly soluble particles differs between rats and humans. Nikula *et al*. (2001) [ADDIN EN.CITE <EndNote><Cite><Author>Nikula</Author><Year>2001</Year><RecNum>62</RecNum><DisplayText>[54]</DisplayText><record><rec-number>62</rec-number><foreign-keys><key app="EN" db-id="xs0a90va7aasfwex5aev0dvyp0t59sta5dae" timestamp="1595803440">62</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Nikula, K. J.</author><author>Vallyathan, V.</author><author>Green, F. H.</author><author>Hahn, F. F.</author></authors></contributors><auth-address>Lovelace Respiratory Research Institute, Albuquerque, New Mexico 87185, USA.</auth-address><titles><title>Influence of exposure concentration or dose on the distribution of particulate material in rat and human lungs</title><secondary-title>Environ Health Perspect</secondary-title><alt-title>Environmental health perspectives</alt-title></titles><periodical><full-title>Environ Health Perspect</full-title></periodical><pages>311-8</pages><volume>109</volume><number>4</number><edition>2001/05/04</edition><keywords><keyword>Adult</keyword><keyword>Air Pollutants/*pharmacokinetics</keyword><keyword>Animals</keyword><keyword>Coal</keyword><keyword>Dose-Response Relationship, Drug</keyword><keyword>Dust</keyword><keyword>Humans</keyword><keyword>*Inhalation Exposure</keyword><keyword>Lung/*chemistry</keyword><keyword>Macrophages, Alveolar</keyword><keyword>Male</keyword><keyword>Middle Aged</keyword><keyword>*Mining</keyword><keyword>*Occupational

Exposure</keyword><keyword>Particle Size</keyword><keyword>Rats</keyword><keyword>Rats, Inbred F344</keyword><keyword>Vehicle Emissions/*analysis</keyword></keywords><dates><year>2001</year><pub-dates><date>Apr</date></pub-dates></dates><isbn>0091-6765 (Print)
0091-6765</isbn><accession-num>11335177</accession-num><urls></urls><custom2>PMC1240269</custom2><electronic-resource-num>10.1289/ehp.01109311</electronic-resource-num><remote-database-provider>NLM</remote-database-provider><language>eng</language></record></Cite></EndNote>] showed that "the relative amounts of intraluminal and interstitial particle load differ markedly between rats and humans with particles being found predominantly in the interstitium in man and intra-luminarly in rats." In rats, accumulation of particulate matter in the intraluminal space leads to adverse "alveolar epithelial hyperplastic, inflammatory, and septal fibrotic responses" [ADDIN EN.CITE <EndNote><Cite><Author>ECETOC</Author><Year>2013</Year><RecNum>9</RecNum><DisplayText>[29]</DisplayText><record><rec-number>9</rec-number><foreign-keys><key app="EN" db-id="xs0a90va7aasfwex5aev0dvyp0t59sta5dae" timestamp="1590845309">9</key></foreign-keys><ref-type name="Report">27</ref-type><contributors><authors><author>ECETOC</author></authors></contributors><titles><title>Poorly Soluble Particles / Lung Overload</title></titles><pages>130, http://www.ecetoc.org/wp-content/uploads/2014/08/ECETOC-TR-122-Poorly-Soluble-Particles-Lung-Overload.pdf</pages><number>Technical Report No. 122</number><dates><year>2013</year><pub-dates><date>December 2013</date></pub-

dates></dates><pub-location>Brussels, Belguim</pub-location><publisher>European Centre for Ecotoxicology and Toxicology of Chemicals</publisher><work-type>Technical Report</work-type><urls><related-urls><url>http://www.ecetoc.org/wp-content/uploads/2014/08/ECETOC-TR-122-Poorly-Soluble-Particles-Lung-Overload.pdf</url></related-urls></urls></record></Cite></EndNote>].

As noted previously, EPA generally uses the polyvinyls sub-category analogue (*i.e.*, PVC powder) POD of 3.3 mg/m^3 for evaluating new chemical substances that may present a lung overload hazard when the chemical properties are comparable between the new chemical substance and the PVC powder. The polyvinyls sub-category POD is then subject to established EPA dosimetry adjustment. Each of these approaches is discussed below. These dosimetric adjustments may also be applied to the polyacrylates/methacrylates sub-category analogue (9000 Toner), as well as to data on new chemical substances or other potential analogues that fit within the chemical boundaries for this category.

As shown in [REF _Ref519678474 \h * MERGEFORMAT], the RDDRs for the PVC powder ranged from 0.501 in the pulmonary region (PU) up to 2.248 in the tracheobronchial (TB) region. Since the effects occurred in the PU region, the PU RDDR was used for deriving a POD_{HEC}, as follows:

$$POD_{HEC} = POD \times RDDR_{PU}$$

or

$$POD_{HEC} = 3.3 \text{ mg/m}^3 \times 0.5 = 1.65 \text{ mg/m}^3$$

Table [SEQ Table * ARABIC]. Depositional fractions and RDDRs for rats and humans.[a]

SPECIES	Extrathoracic (ET)		Tracheobronchial (TB)		Pulmonary (PU)		Thoracic (TB + PU)		Total Respiratory Tract (RT)	
	Surface Area (cm^2)	Depositional Fraction	Surface Area (cm^2)	Depositional Fraction	Surface Area (m^2)	Depositional Fraction	Surface Area (m^2)	Depositional Fraction	Surface Area (m^2)	Depositional Fraction
Rat	15	0.33	22.5	0.068	0.34	0.061	0.342	0.129	0.344	0.459
Human	200	0.24	3200	0.059	54	0.267	54.32	0.125	54.34	0.566
RDD	0.075	1.373	0.007	1.15	0.006	0.229	0.006	1.028	0.006	0.811
RDDR	0.252		2.248		0.501		0.863		1.763	

[a] Inputted values included: MMAD = 1.30; GSD = 2.07.

In comparison, the MPPD model was used to conduct simulations to predict retained mass burden in the PU region of female F344 rats exposed in the Muhle *et al.* (1990) [ADDIN EN.CITE <EndNote><Cite><Author>Muhle</Author><Year>1990</Year><RecNum>13</RecNum><DisplayText>[46]</DisplayText><record><rec-number>13</rec-number><foreign-keys><key app="EN" db-id="xs0a90va7aasfwex5aev0dvyp0t59sta5dae" timestamp="1590845894">13</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Muhle, H.</author><author>Bellmann, B.</author><author>Creutzenberg, O.</author><author>Heinrich, U.</author><author>Ketkar, M.</author><author>Mermelstein, R.</author></authors></contributors><titles><title>Dust overloading of lungs after exposure of rats to particles of low solubility: Comparative studies</title><secondary-title>Journal of Aerosol Science</secondary-title></titles><periodical><full-title>Journal of Aerosol Science</full-title></periodical><pages>374-377</pages><volume>21</volume><number>3</number><dates><year>1990</year></dates><urls></urls><electronic-resource-num>https://doi.org/10.1016/0021-8502(90)90062-3</electronic-resource-num></record></Cite></EndNote>] study. The geometry model in the MPPD software for the Sprague-Dawley rat was used, but with the Agency default body weight (BW) of 229 grams for female F-344 rats in a chronic study [ADDIN EN.CITE <EndNote><Cite><Author>EPA</Author><Year>1994</Year><RecNum>47</RecNum><DisplayText>[15]</DisplayText><record><rec-number>47</rec-number><foreign-keys><key app="EN" db-id="xs0a90va7aasfwex5aev0dvyp0t59sta5dae" timestamp="1595788909">47</key></foreign-keys><ref-type name="Journal Article">17</ref-

Commented [ST7]: Section new based on parts of the write up that Owen and Annie developed. The remaining sections of the write up are in the Supporting File.

type><contributors><authors><author>EPA</author></authors></contributors><titles><title>Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry</title><secondary-title>Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina</secondary-title></titles><periodical><full-title>Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina</full-title></periodical><pages>389, https://www.epa.gov/sites/production/files/2014-11/documents/rfc_methodology.pdf</pages><volume>EP/600/9-90/066F</volume><dates><year>1994</year></dates><urls></urls></record></Cite></EndNote>]. The MPPD software internally scales ventilation parameters and respiratory volumes based on BW, so this resulted in tidal volume (V_T) of 1.54, a breathing frequency of 166 bpm, functional residual capacity (FRC) of 3.01 mL, and an upper respiratory tract (URT) volume of 0.34 mL. The 229 g rat PU surface area is predicted to be 1997 cm^2. The particle MMAD, GSD of the particle size distribution, and its density were: 1.3 μm, 2.07, and 1.3 g/cm^3, respectively. The regimen and duration of the nose-only exposure in the Muhle *et al*. (1990) [ADDIN EN.CITE <EndNote><Cite><Author>Muhle</Author><Year>1990</Year><RecNum>13</RecNum><DisplayText>[46]</DisplayText><record><rec-number>13</rec-number><foreign-keys><key app="EN" db-id="xs0a90va7aasfwex5aev0dvyp0t59sta5dae" timestamp="1590845894">13</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Muhle, H.</author><author>Bellmann, B.</author><author>Creutzenberg, O.</author><author>Heinrich, U.</author><author>Ketkar, M.</author><author>Mermelstein, R.</author></authors></contributors><titles><title>Dust

overloading of lungs after exposure of rats to particles of low solubility: Comparative studies</title><secondary-title>Journal of Aerosol Science</secondary-title></titles><periodical><full-title>Journal of Aerosol Science</full-title></periodical><pages>374-377</pages><volume>21</volume><number>3</number><dates><year>1990</year></dates><urls></urls><electronic-resource-num>https://doi.org/10.1016/0021-8502(90)90062-3</electronic-resource-num></record></Cite></EndNote>] study was 5 h/d and 5 d/w for 8 months and was used in the simulation. We note that there were discrepancies in the reported duration of exposure of 7 months versus 8 months in Muhle *et al.* (1990) [ADDIN EN.CITE <EndNote><Cite><Author>Muhle</Author><Year>1990</Year><RecNum>13</RecNum><DisplayText>[46]</DisplayText><record><rec-number>13</rec-number><foreign-keys><key app="EN" db-id="xs0a90va7aasfwex5aev0dvyp0t59sta5dae" timestamp="1590845894">13</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Muhle, H.</author><author>Bellmann, B.</author><author>Creutzenberg, O.</author><author>Heinrich, U.</author><author>Ketkar, M.</author><author>Mermelstein, R.</author></authors></contributors><titles><title>Dust overloading of lungs after exposure of rats to particles of low solubility: Comparative studies</title><secondary-title>Journal of Aerosol Science</secondary-title></titles><periodical><full-title>Journal of Aerosol Science</full-title></periodical><pages>374-377</pages><volume>21</volume><number>3</number><dates><year>1990</year></dates><urls></urls><electronic-resource-num>https://doi.org/10.1016/0021-8502(90)90062-3</electronic-resource-num></record></Cite></EndNote>]. However, the Bellmann *et al.*

(1986) [ADDIN EN.CITE <EndNote><Cite><Author>Bellmann</Author><Year>1986</Year><RecNum>77</RecNum><DisplayText>[55]</DisplayText><record><rec-number>77</rec-number><foreign-keys><key app="EN" db-id="xs0a90va7aasfwex5aev0dvyp0t59sta5dae" timestamp="1595887108">77</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Bellmann, B.</author><author>Muhle, H.</author><author>Creutzenberg, O.</author></authors></contributors><titles><title>The Effect of a "Nuisance" Dust Inhalation of Lung Clearance</title><secondary-title>Aerosols, Formation and Reactivity, Proceedings of the Second International Aerosol Conference</secondary-title></titles><periodical><full-title>Aerosols, Formation and Reactivity, Proceedings of the Second International Aerosol Conference</full-title></periodical><pages>209-211</pages><dates><year>1986</year></dates><urls></urls></record></Cite></EndNote>] abstract consistently reported an 8-month exposure duration; therefore, a duration of 8-months was used.

Using the above experimental conditions, the predicted retained mass in the PU region of F344 rats, shown in [REF _Ref46766078 \h * MERGEFORMAT], demonstrated the fit of the MPPD model to the experimental data reported by Muhle *et al.* (1990) [ADDIN EN.CITE <EndNote><Cite><Author>Muhle</Author><Year>1990</Year><RecNum>13</RecNum><DisplayText>[46]</DisplayText><record><rec-number>13</rec-number><foreign-keys><key app="EN" db-id="xs0a90va7aasfwex5aev0dvyp0t59sta5dae" timestamp="1590845894">13</key></foreign-keys><ref-type name="Journal Article">17</ref-

type><contributors><authors><author>Muhle, H.</author><author>Bellmann, B.</author><author>Creutzenberg, O.</author><author>Heinrich, U.</author><author>Ketkar, M.</author><author>Mermelstein, R.</author></authors></contributors><titles><title>Dust overloading of lungs after exposure of rats to particles of low solubility: Comparative studies</title><secondary-title>Journal of Aerosol Science</secondary-title></titles><periodical><full-title>Journal of Aerosol Science</full-title></periodical><pages>374-377</pages><volume>21</volume><number>3</number><dates><year>1990</year></dates><urls></urls><electronic-resource-num>https://doi.org/10.1016/0021-8502(90)90062-3</electronic-resource-num></record></Cite></EndNote>]. Additional simulations were conducted using the same three exposure concentration as Muhle *et al.* (1990) [ADDIN EN.CITE <EndNote><Cite><Author>Muhle</Author><Year>1990</Year><RecNum>13</RecNum><DisplayText>[46]</DisplayText><record><rec-number>13</rec-number><foreign-keys><key app="EN" db-id="xs0a90va7aasfwex5aev0dvyp0t59sta5dae" timestamp="1590845894">13</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Muhle, H.</author><author>Bellmann, B.</author><author>Creutzenberg, O.</author><author>Heinrich, U.</author><author>Ketkar, M.</author><author>Mermelstein, R.</author></authors></contributors><titles><title>Dust overloading of lungs after exposure of rats to particles of low solubility: Comparative studies</title><secondary-title>Journal of Aerosol Science</secondary-title></titles><periodical><full-title>Journal of Aerosol Science</full-title></periodical><pages>374-

377</pages><volume>21</volume><number>3</number><dates><year>1990</year></dates>
<urls></urls><electronic-resource-num>https://doi.org/10.1016/0021-8502(90)90062-
3</electronic-resource-num></record></Cite></EndNote>], but the key input parameters for MMAD, GSD, and density were varied and bounded. Details on the additional simulations are provided under "Section 4 MPPD Modeling Outputs" of the Supporting Information file.

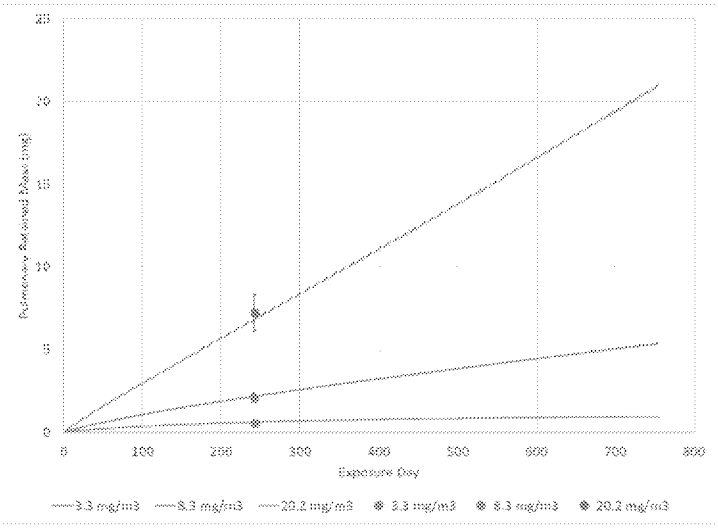

Figure [SEQ Figure * ARABIC]. MPPD predictions for retained PU mass in F344 rats under the exposure conditions for the Muhle et al. (1990) [ADDIN EN.CITE
<EndNote><Cite><Author>Muhle</Author><Year>1990</Year><RecNum>13</RecNum><Di
splayText>[46]</DisplayText><record><rec-number>13</rec-number><foreign-keys><key
app="EN" db-id="xs0a90va7aasfwex5aev0dvyp0t59sta5dae"
timestamp="1590845894">13</key></foreign-keys><ref-type name="Journal Article">17</ref-

[PAGE]

type><contributors><authors><author>Muhle, H.</author><author>Bellmann, B.</author><author>Creutzenberg, O.</author><author>Heinrich, U.</author><author>Ketkar, M.</author><author>Mermelstein, R.</author></authors></contributors><titles><title>Dust overloading of lungs after exposure of rats to particles of low solubility: Comparative studies</title><secondary-title>Journal of Aerosol Science</secondary-title></titles><periodical><full-title>Journal of Aerosol Science</full-title></periodical><pages>374-377</pages><volume>21</volume><number>3</number><dates><year>1990</year></dates><urls></urls><electronic-resource-num>https://doi.org/10.1016/0021-8502(90)90062-3</electronic-resource-num></record></Cite></EndNote>] study. Simulations were performed to characterize the 8-month study with a particle MMAD size of 1.3 μm, a GSD of 2.07, and a density of 1.3 g/cm^3 for three concentrations (3.3, 8.3, and 20.2 mg/m^3). Experimental data for PU burdens are shown as solid circles with standard deviation and the predictions as solid lines for different concentrations.

For extrapolation of the predicted rat retained PU mass to an HEC, human simulations were conducted for adult males with a V_T of 0.992 L and a breathing frequency of 21 bpm, or with 1.364 L and 33 bpm. These ventilatory values are from the ICRP (1994) [ADDIN EN.CITE <EndNote><Cite><Author>ICRP</Author><Year>1994</Year><RecNum>26</RecNum><DisplayText>[20]</DisplayText><record><rec-number>26</rec-number><foreign-keys><key app="EN" db-id="xs0a90va7aasfwex5aev0dvyp0t59sta5dae" timestamp="1590848620">26</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>ICRP</author></authors></contributors><titles><title>

Human respiratory tract model for radiological protection. A report of a Task Group of the International Commission on Radiological Protection</title><secondary-title>Ann ICRP</secondary-title><alt-title>Annals of the ICRP</alt-title></titles><periodical><full-title>Ann ICRP</full-title><abbr-1>Annals of the ICRP</abbr-1></periodical><alt-periodical><full-title>Ann ICRP</full-title><abbr-1>Annals of the ICRP</abbr-1></alt-periodical><pages>1-482</pages><volume>24</volume><number>1-3</number><edition>1994/01/01</edition><keywords><keyword>Humans</keyword><keyword>International Cooperation</keyword><keyword>*Models, Theoretical</keyword><keyword>Neoplasms, Radiation-Induced/*etiology/pathology/physiopathology</keyword><keyword>Radiation Dosage</keyword><keyword>*Radiation Monitoring</keyword><keyword>*Radiation Protection</keyword><keyword>Radioactive Pollutants</keyword><keyword>Respiratory System/pathology/physiopathology/*radiation effects</keyword><keyword>Respiratory Tract Neoplasms/*etiology/pathology/physiopathology</keyword></keywords><dates><year>1994</year></dates><isbn>0146-6453 (Print)
0146-6453</isbn><accession-num>7726471</accession-num><urls><related-urls><url>https://journals.sagepub.com/doi/pdf/10.1177/ANIB_24_1-3</url></related-urls></urls><remote-database-provider>NLM</remote-database-provider><language>eng</language></record></Cite></EndNote>] and represent ventilation associated with activity levels of either light exercise or heavy exercise for adult males. It should be noted that this combination of V_T and bpm for the light exercise ventilation input parameters are equivalent to the default minute ventilation value (V_E) found in [REF _Ref46666189 \h *

MERGEFORMAT] of 1.25 m^3/hr. An occupational exposure duration of 40 years was simulated for the human predictions of retained mass in the PU region.

The dose metric used to operationally derive the HEC is the PU retained mass (mg) normalized to the PU surface area (SA) in cm^2 according to the established US EPA methods [ADDIN EN.CITE <EndNote><Cite><Author>EPA</Author><Year>1994</Year><RecNum>47</RecNum><DisplayText>[15]</DisplayText><record><rec-number>47</rec-number><foreign-keys><key app="EN" db-id="xs0a90va7aasfwex5aev0dvyp0t59sta5dae" timestamp="1595788909">47</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry</title><secondary-title>Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina</secondary-title></titles><periodical><full-title>Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina</full-title></periodical><pages>389, https://www.epa.gov/sites/production/files/2014-11/documents/rfc_methodology.pdf</pages><volume>EP/600/9-90/066F</volume><dates><year>1994</year></dates><urls></urls></record></Cite></EndNote>]. The MPPD model estimates a human pulmonary surface area of 66.3 m^2 for an 80 kg adult male. As shown in [REF _Ref46767442 \h * MERGEFORMAT], simulations were performed iteratively to arrive at an HEC that achieved the same internal dose metric (PU mass / PU SA) in humans as was achieved in rats under the experimental conditions reported by Muhle *et al.*

(1990) [ADDIN EN.CITE <EndNote><Cite><Author>Muhle</Author><Year>1990</Year><RecNum>13</RecNum><DisplayText>[46]</DisplayText><record><rec-number>13</rec-number><foreign-keys><key app="EN" db-id="xs0a90va7aasfwex5aev0dvyp0t59sta5dae" timestamp="1590845894">13</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Muhle, H.</author><author>Bellmann, B.</author><author>Creutzenberg, O.</author><author>Heinrich, U.</author><author>Ketkar, M.</author><author>Mermelstein, R.</author></authors></contributors><titles><title>Dust overloading of lungs after exposure of rats to particles of low solubility: Comparative studies</title><secondary-title>Journal of Aerosol Science</secondary-title></titles><periodical><full-title>Journal of Aerosol Science</full-title></periodical><pages>374-377</pages><volume>21</volume><number>3</number><dates><year>1990</year></dates><urls></urls><electronic-resource-num>https://doi.org/10.1016/0021-8502(90)90062-3</electronic-resource-num></record></Cite></EndNote>]. As was shown in [REF _Ref46766078 \h * MERGEFORMAT], the predicted retained mass in the PU region corresponds well with the observed experimental data. The last two rows of [REF _Ref46767442 \h * MERGEFORMAT] demonstrate the difference in HEC value due to variation in ventilatory parameters associated with either light or heavy activity.

Table [SEQ Table * ARABIC]. MPPD predictions and HEC calculations for Muhle *et al.* (1990) study of F344 rats exposed to PVC with a particle MMAD of 1.3 μm, GSD of 2.07 and density of 1.3 gm / cm^3.

Exposure Concentration (mg/m^3)	**3.3**	**8.3**	**20.2**
Experimental Rat Retained PU Mass (mg)	0.56±0.16	2.09±0.29	7.24±1.10

[PAGE]

Predicted Rat Retained PU Mass (mg)	0.63	2.21	6.88
Predicted Rat Retained PU Mass / PU SA (mg/m^2)	2.8	10.5	36.3
Light Activity 40-Year HEC (mg/m^3)	0.33	1.23	4.25
Heavy Activity 40-Year HEC (mg/m^3)	0.14	0.53	1.84

HEC = human equivalent concentration that results in the same inhaled dose metric (retained PU mass / PU SA) as predicted for the rat. The human ventilatory parameters of the light and heavy activity levels for simulation of 40-year occupational scenario are described in the text.

Category benchmark margin of exposure (MOE)

EPA currently applies a composite UF of 1,000 as the benchmark MOE for the PVC powder POD of 3.3 mg/m^3. The composite UF consists of default values of 10 for UF_H, UF_A, and UF_L. This default approach was initially established as a conservative means of evaluating new chemistries on HMW polymers, which were anticipated to present a hazard concern for lung overload. However, several refinements to these values may be made, including reducing the TK and TD components of the UF_A value and reducing the UF_L. Dosimetric adjustments using the RDDR model or the MPPD model, as discussed above, may be applied to calculate a POD_{HEC}, thereby reducing the TK component of the UF_A to 1. Since lung overload is a chronic effect that is manifested primarily based on the retained dose, the RDDR model is not necessarily the most appropriate for deriving a POD_{HEC}, given that deposition is a more relevant metric for short-term effects/exposures. However, the RDDR model was used to provide comparative estimates of the MOE to the other approaches versus the respective benchmark MOE, given that the RDDR approach is recommended in EPA guidance for quantifying POD_{HECs} for particles. For the TD component, a reduced value of 1 may be applied based on the proposal from the ILSI Workshop Consensus Report on rat lung response to particle overload, which stated: "For both neoplastic and fibrogenic endpoints in the rat, associated with PSP exposures, the work group proposed that

the TD component of the interspecies UF be reduced from a factor of 3 to 1, given that chronic active inflammation in the rat appears to be a more sensitive response than in other species, including humans" [ADDIN EN.CITE ADDIN EN.CITE.DATA]. The UF_L may be reduced from 10 to 1 for the PVC powder analogue POD because this dose represented the point at which retardation of alveolar clearance started, based on the retained mass of about 0.5 mg/lung. This approach is consistent with EPA (2002) [ADDIN EN.CITE <EndNote><Cite><Author>EPA</Author><Year>2002</Year><RecNum>46</RecNum><DisplayText>[14]</DisplayText><record><rec-number>46</rec-number><foreign-keys><key app="EN" db-id="xs0a90va7aasfwex5aev0dvyp0t59sta5dae" timestamp="1595788591">46</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>A Review of the Reference Dose and Reference Concentration Processes</title><secondary-title>Risk Assessment Forum, U.S. Environmental Protection Agency, Washington, DC 20460</secondary-title></titles><periodical><full-title>Risk Assessment Forum, U.S. Environmental Protection Agency, Washington, DC 20460</full-title></periodical><pages>192, https://www.epa.gov/sites/production/files/2014-12/documents/rfd-final.pdf</pages><volume>EPA/630/P-02/002F</volume><dates><year>2002</year></dates><urls></urls></record></Cite></EndNote>], which states that the UF_L "may be altered, depending on the magnitude and nature of the response at the LOAEL". Further, the default application of this UF is for apical endpoints, rather than initial key events in an adverse outcome pathway. Based on the foregoing considerations, the following values are proposed for deriving the benchmark MOE for HMW

polymers, which are generally applicable regardless of whether the POD is derived from an analogue or a new chemical substance.

UF_H = 10: The default value of 10 should be applied, unless there are human data showing which age groups or time periods are the most sensitive to lung overload. This approach is consistent with EPA's guidance for reducing the default UF_H [ADDIN EN.CITE <EndNote><Cite><Author>EPA</Author><Year>2002</Year><RecNum>46</RecNum><DisplayText>[14]</DisplayText><record><rec-number>46</rec-number><foreign-keys><key app="EN" db-id="xs0a90va7aasfwex5aev0dvyp0t59sta5dae" timestamp="1595788591">46</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>A Review of the Reference Dose and Reference Concentration Processes</title><secondary-title>Risk Assessment Forum, U.S. Environmental Protection Agency, Washington, DC 20460</secondary-title></titles><periodical><full-title>Risk Assessment Forum, U.S. Environmental Protection Agency, Washington, DC 20460</full-title></periodical><pages>192, https://www.epa.gov/sites/production/files/2014-12/documents/rfd-final.pdf</pages><volume>EPA/630/P-02/002F</volume><dates><year>2002</year></dates><urls></urls></record></Cite></EndNote>].

UF_A = 3 or 1: A reduced value of 1 should be applied for the TD component based on the proposal documented by Olin (2000). In addition, if the data are amenable for deriving a POD_{HEC}, the dosimetric adjustment for the TK component further supports reducing this UF [

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<EndNote><Cite><Author>EPA</Author><Year>2002</Year><RecNum>46</RecNum><DisplayText>[14, 15]</DisplayText><record><rec-number>46</rec-number><foreign-keys><key app="EN" db-id="xs0a90va7aasfwex5aev0dvyp0t59sta5dae" timestamp="1595788591">46</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>A Review of the Reference Dose and Reference Concentration Processes</title><secondary-title>Risk Assessment Forum, U.S. Environmental Protection Agency, Washington, DC 20460</secondary-title></titles><periodical><full-title>Risk Assessment Forum, U.S. Environmental Protection Agency, Washington, DC 20460</full-title></periodical><pages>192, https://www.epa.gov/sites/production/files/2014-12/documents/rfd-final.pdf</pages><volume>EPA/630/P-02/002F</volume><dates><year>2002</year></dates><urls></urls></record></Cite><Cite><Author>EPA</Author><Year>1994</Year><RecNum>47</RecNum><record><rec-number>47</rec-number><foreign-keys><key app="EN" db-id="xs0a90va7aasfwex5aev0dvyp0t59sta5dae" timestamp="1595788909">47</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry</title><secondary-title>Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina</secondary-title></titles><periodical><full-title>Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina</full-

title></periodical><pages>389, https://www.epa.gov/sites/production/files/2014-11/documents/rfc_methodology.pdf</pages><volume>EP/600/9-90/066F</volume><dates><year>1994</year></dates><urls></urls></record></Cite></EndNote>].

UF_L = 10 or 1: A value of 1 should be applied when the POD is based on a study NOAEC or when BMD modeling is applied to derive a BMCL, per EPA guidance [ADDIN EN.CITE <EndNote><Cite><Author>EPA</Author><Year>2012</Year><RecNum>49</RecNum><DisplayText>[22]</DisplayText><record><rec-number>49</rec-number><foreign-keys><key app="EN" db-id="xs0a90va7aasfwex5aev0dvyp0t59sta5dae" timestamp="1595789576">49</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>Benchmark Dose Technical Guidance</title><secondary-title>Risk Assessment Forum, U.S. Environmental Protection Agency, Washington, DC 20460</secondary-title></titles><periodical><full-title>Risk Assessment Forum, U.S. Environmental Protection Agency, Washington, DC 20460</full-title></periodical><pages>99, https://www.epa.gov/sites/production/files/2015-01/documents/benchmark_dose_guidance.pdf</pages><volume>EPA/100/R-12/001</volume><dates><year>2012</year></dates><urls></urls></record></Cite></EndNote>]. The default value of 10 should be applied when the POD is based on a study LOAEC; however, a reduced value may be used, when for example, the LOAEC is based on key event 1 from the proposed adverse outcome pathway for PSPs. Reductions in the UF_L based on other key

events should be made on a case-by-case basis and supported by discussion of the key event within the context of an established AOP.

The default and dosimetrically adjusted PODs and benchmark MOEs derived on new chemical substance risk assessments are used to inform risk management options for addressing potential risks. For example, the default POD of 3.3 mg/m^3 and benchmark MOE of 1,000 result in an MOE of 2.0E-01 that would require engineering controls and/or a respirator with an applied protection factor (APF) of 1,000. In comparison, when dosimetric adjustments are applied using the MPPD modeling outputs, the $POD_{HEC\text{-}light\ activity}$ of 0.33 mg/m^3 and refined benchmark MOE of 10 result in an MOE 1.7, which indicates that engineering controls and/or a respirator with an APF of 10 would be required.

Uncertainties and Limitations

The available toxicological studies for HMW polymers lack data on materials with molecular weights < 70,000 Daltons [ADDIN EN.CITE <EndNote><Cite><Author>EPA</Author><Year>2020</Year><RecNum>63</RecNum><DisplayText>[57]</DisplayText><record><rec-number>63</rec-number><foreign-keys><key app="EN" db-id="xs0a90va7aasfwex5aev0dvyp0t59sta5dae" timestamp="1595803909">63</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>High Molecular Weight Polymers in the New Chemicals Program</title><secondary-title>Office of Pollution Prevention and Toxics, U.S. Environmental Protection Agency, 1200 Pennsylvania Ave., NW, Washington, DC 20460</secondary-title></titles><periodical><full-title>Office of

Pollution Prevention and Toxics, U.S. Environmental Protection Agency, 1200 Pennsylvania Ave., NW, Washington, DC 20460</full-title></periodical><pages>https://www.epa.gov/reviewing-new-chemicals-under-toxic-substances-control-act-tsca/high-molecular-weight-polymers-new</pages><dates><year>2020</year></dates><urls></urls></record></Cite></EndNote>]. In addition, the following uncertainties and study limitations were noted, that if known, may serve to refine the boundaries for this category:

- Physicochemical properties can influence deposition of inhaled particles (*e.g.*, particle size, distribution, density, and hygroscopicity) and biopersistence and bioreactivity (*e.g.*, solubility, surface chemistry, and composition). However, the available studies of test materials in this category are generally missing information on these properties, with the exception of particle size.
- Information on molecular weight was not reported for test materials used in the studies of the PVC powder [ADDIN EN.CITE <EndNote><Cite><Author>Muhle</Author><Year>1990</Year><RecNum>13</RecNum><DisplayText>[46]</DisplayText><record><rec-number>13</rec-number><foreign-keys><key app="EN" db-id="xs0a90va7aasfwex5aev0dvyp0t59sta5dae" timestamp="1590845894">13</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Muhle, H.</author><author>Bellmann, B.</author><author>Creutzenberg, O.</author><author>Heinrich, U.</author><author>Ketkar,

M.</author><author>Mermelstein,

R.</author></authors></contributors><titles><title>Dust overloading of lungs after exposure of rats to particles of low solubility: Comparative studies</title><secondary-title>Journal of Aerosol Science</secondary-title></titles><periodical><full-title>Journal of Aerosol Science</full-title></periodical><pages>374-377</pages><volume>21</volume><number>3</number><dates><year>1990</year></dates><urls></urls><electronic-resource-num>https://doi.org/10.1016/0021-8502(90)90062-3</electronic-resource-num></record></Cite></EndNote>].

- The test materials administered in the 9000 toner studies [ADDIN EN.CITE ADDIN EN.CITE.DATA] included colorant materials (predominantly carbon black) at up to 10%, and the influence of these colorants on the observed effects is unknown.
- The PODs summarized in [REF _Ref46678612 \h * MERGEFORMAT] for the HMW polymers were reported on a mass/volume basis. However, there is evidence that number of particles, particle volume, and/or volume of particles retained in the lung can influence the threshold at which lung overload conditions occur [ADDIN EN.CITE ADDIN EN.CITE.DATA]. Thus, particle density may be an important consideration in identifying a POD; however, the appropriate density metric and how density should be incorporated remain uncertain [ADDIN EN.CITE <EndNote><Cite><Author>ECETOC</Author><Year>2013</Year><RecNum>9</RecNum><DisplayText>[29]</DisplayText><record><rec-number>9</rec-number><foreign-keys><key app="EN" db-id="xs0a90va7aasfwex5aev0dvyp0t59sta5dae" timestamp="1590845309">9</key></foreign-keys><ref-type name="Report">27</ref-

type><contributors><authors><author>ECETOC</author></authors></contributors><tit les><title>Poorly Soluble Particles / Lung Overload</title></titles><pages>130, http://www.ecetoc.org/wp-content/uploads/2014/08/ECETOC-TR-122-Poorly-Soluble-Particles-Lung-Overload.pdf</pages><number>Technical Report No. 122</number><dates><year>2013</year><pub-dates><date>December 2013</date></pub-dates></dates><pub-location>Brussels, Belguim</pub-location><publisher>European Centre for Ecotoxicology and Toxicology of Chemicals</publisher><work-type>Technical Report</work-type><urls><related-urls><url>http://www.ecetoc.org/wp-content/uploads/2014/08/ECETOC-TR-122-Poorly-Soluble-Particles-Lung-Overload.pdf</url></related-urls></urls></record></Cite></EndNote>].

- Particle morphology, reactive groups, and cytotoxicity can impede clearance pathways and induce other mechanisms of toxicity in rodents and humans. These factors include covalent binding to lung tissues, toxicity to clearance macrophages/cilia and particles lodging in pulmonary tissues which may not be considered in aerodynamic models. An *in vitro* macrophage clearance assay utilizing human or primate cells and rat cells would be potentially useful information to determine whether new chemistries fall within or outside the boundaries for this category.

An additional, important consideration pertains to the uncertainty associated with the human relevance of lung tumors observed in rats exposed to PSPs. The available data clearly demonstrate that the rat is a sensitive model for non-neoplastic pulmonary effects following repeated exposure to PSPs, which have also been shown to occur in occupational cohorts (*e.g.*,

coal miners). The rat also appears to be unique among species with regard to carcinogenesis due to particle overload. Lung tumors following chronic exposure to PSPs have been reported in rats, but have not been reported in mice, hamster, non-human primates, or humans [ADDIN EN.CITE <EndNote><Cite><Author>ECETOC</Author><Year>2013</Year><RecNum>9</RecNum><DisplayText>[29]</DisplayText><record><rec-number>9</rec-number><foreign-keys><key app="EN" db-id="xs0a90va7aasfwex5aev0dvyp0t59sta5dae" timestamp="1590845309">9</key></foreign-keys><ref-type name="Report">27</ref-type><contributors><authors><author>ECETOC</author></authors></contributors><titles><title>Poorly Soluble Particles / Lung Overload</title></titles><pages>130, http://www.ecetoc.org/wp-content/uploads/2014/08/ECETOC-TR-122-Poorly-Soluble-Particles-Lung-Overload.pdf</pages><number>Technical Report No. 122</number><dates><year>2013</year><pub-dates><date>December 2013</date></pub-dates></dates><pub-location>Brussels, Belguim</pub-location><publisher>European Centre for Ecotoxicology and Toxicology of Chemicals</publisher><work-type>Technical Report</work-type><urls><related-urls><url>http://www.ecetoc.org/wp-content/uploads/2014/08/ECETOC-TR-122-Poorly-Soluble-Particles-Lung-Overload.pdf</url></related-urls></urls></record></Cite></EndNote>]. Despite the uncertainty in the carcinogenicity of inhaled PSPs, the rat model remains a useful model for lung overload because it is a sensitive model for inflammatory response to PSPs, and because protecting against inflammation and proliferation may also protect against tumor formation [ADDIN EN.CITE ADDIN EN.CITE.DATA].

Tiered-testing Strategy

The POD and benchmark MOE derived herein provide an analogue/read-across approach for assessing new chemical substances that fit within the chemical category boundaries for HMW polymers, also defined herein. As with any analogue read-across, assessors must carefully consider the comparability of the new chemical substance to the analogue or another acceptable toxicological analogue; this framework provides specific criteria for evaluating whether a new chemical substance "fits" into the HMW polymer category (*i.e.*, not chemically reactive, insoluble in water, not expected to be directly cytotoxic, not expected to release toxic degradates). When information is not available to evaluate whether the new chemical substance fits within the category boundaries and the analogue is appropriate for use in a risk assessment, testing should be performed to aid with refining the evaluation of new chemistries that are anticipated to present a potential lung overload hazard. A tiered-testing strategy that is consistent with the reduced vertebrate testing requirements under the amended TSCA is provided. Though this strategy does not completely exclude vertebrate testing, it maximizes the use of NAMs for determining whether vertebrate testing should be considered. This strategy incorporates *in chemico* and/or *in vitro* characterization of the chemical substance in Tier I (*e.g.*, particle size distribution, reactivity, and biosolubility measurements). For substances that have particles in the respirable range, are non-reactive, and are not biosoluble, computational screening is included under Tier II to determine whether the HMW polymer is estimated to exceed the clearance t½ in the rat. If the HMW polymer is expected to exceed the clearance t½ in the rat, then risk management options or strategic *in vivo* testing is proposed as a final option under Tier III.

Tier I

- Particle Size Distribution or Aerosolized Droplet Size of particle in use (*i.e.*, cascade impactor, laser methods, *e.g.*, OECD TG 110 [ADDIN EN.CITE <EndNote><Cite><Author>OECD</Author><Year>1981</Year><RecNum>64</RecNum><DisplayText>[59]</DisplayText><record><rec-number>64</rec-number><foreign-keys><key app="EN" db-id="xs0a90va7aasfwex5aev0dvyp0t59sta5dae" timestamp="1595804668">64</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>OECD</author></authors></contributors><titles><title>Particle Size Distribution/Fibre Length and Diameter Distributions</title><secondary-title>OECD Guideline for Testing of Chemicals</secondary-title></titles><periodical><full-title>OECD Guideline for Testing of Chemicals</full-title></periodical><pages>13, https://www.oecd-ilibrary.org/environment/test-no-110-particle-size-distribution-fibre-length-and-diameter-distributions_9789264069688-en</pages><volume>110</volume><dates><year>1981</year></dates><urls></urls></record></Cite></EndNote>], OPPTS 830.7520 [ADDIN EN.CITE <EndNote><Cite><Author>EPA</Author><Year>1996</Year><RecNum>65</RecNum><DisplayText>[60]</DisplayText><record><rec-number>65</rec-number><foreign-keys><key app="EN" db-id="xs0a90va7aasfwex5aev0dvyp0t59sta5dae" timestamp="1595804850">65</key></foreign-keys><ref-type name="Journal Article">17</ref-

type><contributors><authors><author>EPA</author></authors></contributors><titles><title>Particle Size, Fiber Length, and Diameter Distribution</title><secondary-title>Office of Pollution Prevention and Toxics, U.S. Environmental Protection Agency, 1200 Pennsylvania Ave., NW, Washington, DC 20460</secondary-title></titles><periodical><full-title>Office of Pollution Prevention and Toxics, U.S. Environmental Protection Agency, 1200 Pennsylvania Ave., NW, Washington, DC 20460</full-title></periodical><pages>13, https://www.epa.gov/test-guidelines-pesticides-and-toxic-substances/series-830-product-properties-test-guidelines</pages><volume>EPA 712-C-96-037</volume><dates><year>1996</year></dates><urls></urls></record></Cite></EndNote>]) of the new chemical substance during specific use(s) (*i.e.*, depending on the intended or known uses of the chemical substances, particle size distribution may need to be tested under more than one use scenario)

  - If the % of respirable particles (*i.e.*, $\leq$ 10 μm) is less than 1 wt% under the conditions of use, or following transport, stop at Tier I.
  - If the % of respirable particles (*i.e.*, $\leq$ 10 μm) is greater than 1 wt% under the conditions of use, or if respirable particles are anticipated or shown to be generated following transport (> 1%), then proceed with reactivity testing, if needed, or biosolubility testing.

- Reactivity
  - If the HMW polymer is a potential concern for reactivity, based on function or other information (*e.g.*, does not meet the E1 FG/FGEW criteria), reactivity should be assessed using an *in vitro* method, preferably discussed with EPA in a

pre-notice consultation meeting and prior to study initiation. The assay developed by Wiemann *et al.* (2013) [ADDIN EN.CITE ADDIN EN.CITE.DATA] provides a potential option; however, there are caveats with its use, such as not being validated and uncertainty with whether the test method could be used with HMW polymers, underscoring the recommendation to consult with EPA prior to testing using this method or other test methods.

- If substance is "reactive" (*e.g.*, does not met the E1 FG/FGEW criteria) or based on data from the identified assay or any other appropriate assay, it would be excluded from the HMW polymer category. If evidence indicates the substance is "non-reactive" (*e.g.*, it does meet the E1 FG/FGEW criteria) or based on data from the identified assay or any other appropriate assay, then proceed to biosolubility testing.

- Biosolubility Testing
  - Solubility in Gamble's solution (*e.g.*, ECETOC, 2013 [ADDIN EN.CITE <EndNote><Cite><Author>ECETOC</Author><Year>2013</Year><RecNum>9</RecNum><DisplayText>[29]</DisplayText><record><rec-number>9</rec-number><foreign-keys><key app="EN" db-id="xs0a90va7aasfwex5aev0dvyp0t59sta5dae" timestamp="1590845309">9</key></foreign-keys><ref-type name="Report">27</ref-type><contributors><authors><author>ECETOC</author></authors></contributors><titles><title>Poorly Soluble Particles / Lung

Overload</title></titles><pages>130, http://www.ecetoc.org/wp-content/uploads/2014/08/ECETOC-TR-122-Poorly-Soluble-Particles-Lung-Overload.pdf</pages><number>Technical Report No. 122</number><dates><year>2013</year><pub-dates><date>December 2013</date></pub-dates></dates><pub-location>Brussels, Belguim</pub-location><publisher>European Centre for Ecotoxicology and Toxicology of Chemicals</publisher><work-type>Technical Report</work-type><urls><related-urls><url>http://www.ecetoc.org/wp-content/uploads/2014/08/ECETOC-TR-122-Poorly-Soluble-Particles-Lung-Overload.pdf</url></related-urls></urls></record></Cite></EndNote>]), simulated epithelial lung fluid (SELF) (*e.g.*, Boisa *et al.* 2014 [ADDIN EN.CITE ADDIN EN.CITE.DATA]); and/or phagolysosomal simulant fluid (*e.g.*, BAUA, 2017 [ADDIN EN.CITE <EndNote><Cite><Author>BAUA</Author><Year>2017</Year><RecNum>57</RecNum><DisplayText>[30]</DisplayText><record><rec-number>57</rec-number><foreign-keys><key app="EN" db-id="xs0a90va7aasfwex5aev0dvyp0t59sta5dae" timestamp="1595794599">57</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>BAUA</author></authors></contributors><titles><title>Methodology for the Identification of Granular Biopersistent Particles (GBP) at Workplaces</title><secondary-title>Federal Institute for Occupational Safety and Health</secondary-title></titles><periodical><full-

title>Federal Institute for Occupational Safety and Health</full-title></periodical><pages>103, https://www.baua.de/EN/Service/Publications/Report/F2336.pdf</pages><dates><year>2017</year></dates><urls></urls></record></Cite></EndNote>])

- Employ a simple exponential decay model to predict the dissolution half-life: $P(t)=P0e^{-rt}$, where: P(t) = the amount of some quantity at time t; P0 = initial amount at time t = 0; r = the decay rate; t = time

The exponential decay function is the solution to the first order reaction equation, assuming a constant decay rate, r:

$$\frac{dP(t)}{dt} = -rP(t), P(0) = P_0$$

First order kinetics are used as the basis for lung clearance rates including dissolution and absorption into blood [ADDIN EN.CITE ADDIN EN.CITE.DATA].

- If the solubility data indicate a dissolution rate (*i.e.*, 100 mg/L/day or 72 mg/day) higher than the daily occupational exposure estimate (*e.g.*, default PDR of 50 mg/day), then stop at Tier I.
- If the solubility data indicate a dissolution rate lower than the daily occupational exposure estimate, then proceed with Tier II testing.

If the % of respirable particles is > 1 wt%, the HMW polymer is non-reactive, and the HMW polymer has a dissolution rate that is lower than the estimated daily occupational exposure estimate, proceed to Tier II.

Tier II

- Perform computational modeling (*e.g.*, MPPD) including the effect of dissolution to predict deposition, clearance, and lung burden for a simulated chronic rat exposure (See, *e.g.*, Ladics *et al.*, 2020 [ADDIN EN.CITE <EndNote><Cite><Author>Ladics</Author><Year>2020</Year><RecNum>69</RecNum><DisplayText>[19]</DisplayText><record><rec-number>69</rec-number><foreign-keys><key app="EN" db-id="xs0a90va7aasfwex5aev0dvyp0t59sta5dae" timestamp="1595838584">69</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Ladics, G.</author><author>Price, O.</author><author>Kelkar, S.</author><author>Hermkimer, S.</author><author>Anderson, S.</author></authors></contributors><titles><title>In silico Multiple-Path Particle Dosimetry Modeling of the Lung Burden of a Biosoluble, Bioaccessible Alpha 1,3 Polysaccharide Polymer</title><secondary-title>Chemical Research in Toxicology</secondary-title></titles><periodical><full-title>Chemical Research in Toxicology</full-title></periodical><pages>In preparation</pages><dates><year>2020</year></dates><urls></urls></record></Cite></EndNote>]).
- If the clearance t½ is less than 60 days, stop at Tier II.

If the clearance t½ is greater than that for PSPs in the rat (*i.e.*, 60 days) [ADDIN EN.CITE <EndNote><Cite><Author>Oberdorster</Author><Year>1995</Year><RecNum>60</RecNum

><DisplayText>[36]</DisplayText><record><rec-number>60</rec-number><foreign-keys><key app="EN" db-id="xs0a90va7aasfwex5aev0dvyp0t59sta5dae" timestamp="1595797677">60</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Oberdorster, G.</author></authors></contributors><titles><title>Lung Particle Overload: Implications for Occupational Exposures to Particles</title><secondary-title>Regul Toxicol Pharmacol</secondary-title></titles><periodical><full-title>Regul Toxicol Pharmacol</full-title></periodical><pages>123-135</pages><volume>27</volume><dates><year>1995</year></dates><urls></urls></record></Cite></EndNote>], consider risk management options (*e.g.*, engineering controls and personal protective equipment) or proceed to Tier III.

Tier III

- Strategic *in vivo* testing should be considered, albeit on a case-by-case basis. When performed, the testing should include:
    - Exposure concentrations high enough to demonstrate impaired pulmonary clearance of particles and lead to an "overload" condition. It has been shown that in rats impaired clearance starts when phagocytized particle volume exceeds 6% of normal alveolar macrophage volume and clearance stops altogether when phagocytized volume reaches 60% of normal macrophage volume (See, *e.g.*, Borm *et al*., 2015 [ADDIN EN.CITE ADDIN EN.CITE.DATA]); and
    - Special attention to pulmonary function tests; blood oxygen (pO_2); lung burden measurements and lung clearance kinetics; collection of BALF for assessment of

marker enzyme activities, total protein content, and cell counts; lung retention and clearance; lung weight; and lung histopathology (inflammation and cell proliferation). It is not necessary to evaluate internal organs. OECD TG 413 [ADDIN EN.CITE <EndNote><Cite><Author>OECD</Author><Year>2018</Year><RecNum>71</RecNum><DisplayText>[64]</DisplayText><record><rec-number>71</rec-number><foreign-keys><key app="EN" db-id="xs0a90va7aasfwex5aev0dvyp0t59sta5dae" timestamp="1595838832">71</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>OECD</author></authors></contributors><titles><title>90-Day (Subchronic) Inhalation Toxicity Study</title><secondary-title>OECD Guideline for Testing of Chemicals</secondary-title></titles><periodical><full-title>OECD Guideline for Testing of Chemicals</full-title></periodical><pages>23, https://www.oecd-ilibrary.org/environment/test-no-413-subchronic-inhalation-toxicity-90-day-study_9789264070806-en</pages><volume>413</volume><dates><year>2018</year></dates><urls></urls></record></Cite></EndNote>] and OECD GD 39 [ADDIN EN.CITE <EndNote><Cite><Author>OECD</Author><Year>2018</Year><RecNum>72</RecNum><DisplayText>[65]</DisplayText><record><rec-number>72</rec-number><foreign-keys><key app="EN" db-id="xs0a90va7aasfwex5aev0dvyp0t59sta5dae"

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10/documents/ncp_chemical_categories_august_2010_version_0.pdf</pages><da
tes><year>2010</year></dates><urls></urls></record></Cite></EndNote>].

CONCLUSIONS

The MPPD software provides for a straightforward approach to predict when overload might occur in the experimental species, perform interspecies extrapolation to HEC estimates, and inform inferences for human health risk evaluation. Concentrations at which overload was not achieved in the rat are relevant to human assessment, as are other endpoints other than tumors at overload. Simulations would also be most useful to design of experiments before costly investments in inhalation studies are made and may also help to reduce and refine the number of animals used.

Commented [ST8]: In process

ASSOCIATED CONTENT

Supporting Information.

The Supporting Information file contains the following:

Section 1. Systematic Literature Review

Section 2. Experimental Animal Inhalation Studies on HMW Polymers

Section 3. Benchmark Dose (BMD) Modeling Outputs

Section 4: MPPD Modeling Outputs

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript. ‡These authors contributed equally. (match statement to author names with a symbol)

Funding Sources

EPA sponsored the initial literature review through contract [insert number]. ACC sponsored the supplemental literature review conducted by an independent third party.

Notes

Disclaimer: The views expressed in this article are those of the authors and do not necessarily represent the views or policies of their respective employers. Mention of trade names or commercial products does not constitute endorsement for use.

ACKNOWLEDGMENT

Generally, the last paragraph of the paper is the place to acknowledge people, organizations, and financing (you may state grant numbers and sponsors here).

REFERENCES

[ADDIN EN.REFLIST]

SUPPORTING INFORMATION FOR "POLYMER LUNG OVERLOAD CATEGORY: THE APPLICATION OF NEW APPROACH METHODOLOGIES (NAMS) FOR ASSESSING INHALATION RISKS UNDER THE AMENDED TOXIC SUBSTANCES CONTROL ACT"

1. SYSTEMATIC LITERATURE REVIEW

A. Initial Literature Search

i. Search Strategy

Computerized literature searches were initially conducted in PubMed in November 2016 to obtain studies related to lung overload from inhalation with the intention to identify, in further steps, those relevant for HMW polymers. Since "overload" is defined differently in experimental animals versus humans (Gregoratto et al., 2010, 2011; Kuempel et al., 2001a,b; Sweeney et al., 2013), general MeSH and query terms (*e.g.*, "Lung") and in text words (*e.g.*, "overload") were used with the intent of being overly inclusive. The search query string is presented in [REF _Ref46547342 \h * MERGEFORMAT].

Table [SEQ Table * ARABIC]. PubMed search strategy for lung overload.

Database Search Date	**Query String**[a]
PubMed 11/15/2016	(Aerosols[mh] OR Particulate Matter[mh] OR Dust[mh] OR Lung[mh] OR Lung Diseases/Chemically Induced[mh]) AND Overload[tw]) NOT (Iron[mh]OR Calcium[mh] OR Heart[mh] OR Cardiac[tw])

[a] Note, in the Supplemental Literature Search performed on April 13, 2018, a more comprehensive list of MeSH, query, and text words was included (*e.g.*, "Particle", "Burden", "Retention", "Clearance", *etc.*).

Screening methods for this search included manual screening of titles/abstracts and screening of full text articles using the PECO criteria shown in [REF _Ref46547473 \h * MERGEFORMAT].

Table [SEQ Table * ARABIC]. PECO criteria used to screen literature search results for lung overload

PECO element	**Evidence**[a]
Population	Humans, laboratory animals (rats, mice, hamsters, guinea pigs, dogs, non-human primates, or other inbred mammals) and mammalian cell lines
Exposure	*In vivo* (all routes), *ex vivo* (isolated perfused lung), and *in vitro*
Comparison	Any comparison (across dose, duration, or route) or no comparison (e.g., case reports without controls)
Outcomes	Any examination of: • Pulmonary effects *in vivo* or *ex vivo* studies • Cytotoxicity or alternative methods in *in vitro* studies

[a] The PECO criteria were refined and more specific in the Supplemental Literature Search performed on April 13, 2018 and included, for example, clearance parameters under the PECO element for "Outcomes".

ii. Additional Search Strategies

A search of the gray literature[1] was performed in September 2018 to obtain additional information pertaining to

[1] Gray literature, as used herein, has the same meaning as defined by EPA (2018) and "refers to sources of scientific information that are not formally published and distributed in peer-reviewed journal articles. These references are still valuable and

lung overload from poorly soluble HMW polymers. Resources searched for pertinent gray literature are listed in [REF _Ref46547609 \h * MERGEFORMAT] The chemicals and compound groups identified from the Initial Literature Search and used for gray literature searching are listed in [REF _Ref46547652 \h * MERGEFORMAT]. Screening methods for this search included manual screening of titles/abstracts and full text reports using the PECO criteria shown in [REF _Ref46547473 \h * MERGEFORMAT].

Table [SEQ Table * ARABIC]. List of resources searched for gray literature.

ATSDR [HYPERLINK "http://www.atsdr.cdc.gov/toxprofiles/index.asp"]
Chemtrack [HYPERLINK "http://www.chemtrack.org/White/CMR.pdf"]
CIR [HYPERLINK "http://www.cir-safety.org/ingredients"]
ECETOC publications [HYPERLINK "http://www.ecetoc.org/publications"]
ECHA [HYPERLINK "http://echa.europa.eu/web/guest/information-on-chemicals/registered-substances"]
EFSA (European Food Safety Authority) [HYPERLINK "http://www.efsa.europa.eu/"]
EPA – ChemView (incl. TSCATS data) [HYPERLINK "https://chemview.epa.gov/chemview"]
EPA – HPV Hazard Characterization Documents [HYPERLINK "http://iaspub.epa.gov/oppthpv/hpv_hc_characterization.get_report?doctype=2"]
EPA – HPV Risk-Based Prioritization Documents (RBPs) [HYPERLINK "http://iaspub.epa.gov/oppthpv/hpv_hc_characterization.get_report?doctype=1"]
EPA – HPVIS via ChemID - [HYPERLINK "https://chem.nlm.nih.gov/chemidplus/chemidlite.jsp"]
EPA – TSCATS 1 (available via Toxline)
EPA – pesticides - [HYPERLINK "https://iaspub.epa.gov/apex/pesticides/f?p=CHEMICALSEARCH:1"] Archive [HYPERLINK "https://archive.epa.gov/pesticides/reregistration/web/html/status.html"]
FDA [HYPERLINK "https://www.fda.gov/default.htm"]
HERA [HYPERLINK "http://www.heraproject.com/RiskAssessment.cfm"]
HSDB [HYPERLINK "http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB"]
INCHEM (CICADS, EHC, HSG, IARC, IPCS, JECFA, SIDS) [HYPERLINK "http://www.inchem.org/"]
JECDB (Japan Existing Chemical Data Base) [HYPERLINK "http://dra4.nihs.go.jp/mhlw_data/jsp/SearchPageENG.jsp"]
NICNAS http://www.nicnas.gov.au/
NITE [HYPERLINK "http://www.safe.nite.go.jp/jcheck/search.action?request_locale=en"]
NTP [HYPERLINK "https://ntpsearch.niehs.nih.gov/home"]
OECD [HYPERLINK "http://www.echemportal.org/echemportal/page.action?pageID=9"]
OECD/SIDS [HYPERLINK "http://webnet.oecd.org/hpv/ui/SponsoredChemicals.aspx"]

consulted in the TSCA risk evaluation process. Examples of gray literature are theses and dissertations, technical reports, guideline studies, conference proceedings, publicly-available industry reports, unpublished industry data, trade association resources, and government reports."

Table [SEQ Table * ARABIC]. List of resources searched for gray literature.

ATSDR = Agency for Toxic Substances and Disease Registry; CICADS = Concise International Chemical Assessment Document; CIR = Cosmetic Ingredient Review; ECETOC = European Centre for Ecotoxicology and Toxicology of Chemicals; ECHA = European Chemicals Agency; EFSA = European Food Safety Authority; EHC = Environmental Health Criteria; EPA = Environmental Protection Agency; FDA = Food and Drug Administration; HERA = Human and Environmental Risk Assessment; HPV = High Production Volume; HPVIS = High Production Volume Information System; HSDB = Hazardous Substances Data Bank; HSG = Health and Safety Guideline; IARC = International Agency for Research on Cancer; INCHEM = Internationally Peer Reviewed Chemical Safety Information; IPCS = International Programme on Chemical Safety; JECDB = Japan Existing Chemical Data Base; JEFCA = Joint Expert Committee on Food Additives; NICNAS = National Industrial Chemicals Notification and Assessment Scheme; NITE = National Institute of Technology and Evaluation; NTP =National Toxicology Program; OECD = Organisation for Economic Cooperation and Development; SIDS = Screening Information Data Set; TSCATS = Toxic Substances Control Act Test Submissions

Table [SEQ Table * ARABIC]. Polymer lung overload chemical groups, constituent names, and CASRNs used for searching gray literature.

Chemical Group or Constituent Name	**CASRN**
Styrene/butylmethacrylate random copolymer	25213-39-2[2]
Polyvinyl chloride powder	9002-86-2
Polystyrene spheres	9003-53-6
Linear anionic hexamethylene diisocyanate monomer-based polyurethane-polyurea HMW polymer	No data
Acrylate copolymer	25053-63-8
Butyl acrylate/methacrylic acid polymer	25852-37-3

The reference lists of the primary studies and review articles identified by the PubMed search were manually screened to identify additional pertinent literature for lung overload from HMW polymers (*i.e.*, tree searching). A Supplemental Literature Search was performed in April 2018. The details of this search are provided in the section titled "Supplemental Literature Search". The Supplemental Literature Search was used to identify additional studies or data related to lung overload from HMW polymers that became available after the original search was conducted.

iii. Literature Search and Screening Results

The results of the literature search and screening effort are presented graphically in **[REF _Ref46547725 \h * MERGEFORMAT]**. The PubMed search identified 28 potentially relevant references for full text review. The PubMed search results were supplemented by a review of the reference lists from the relevant publications (*i.e.*, tree searching) which yielded an additional eight references for full text review. An additional four references were obtained from the search of gray literature resources, and the updated literature search identified nine additional references for full text review. Finally, two recent reviews were identified by unstructured PubMed searching.

The full text review of 51 references yielded 24 studies, which consisted of 16 potentially relevant studies with primary data on lung overload from poorly soluble HMW polymers, four studies with supporting information, and four relevant, recent reviews (*i.e.*, references cited in this paper). Twenty-seven articles were excluded. Nineteen of the articles were older or irrelevant reviews. One article, an *in vitro* study, was excluded after full-

[2] The TSCA inventory name for this CASRN is 2-Propenoic acid, 2-methyl-, butyl ester, polymer with ethenylbenzene.

text review (Konczol et al., 2013) because the study authors used a test substance (*i.e.*, carbon-bearing particles covered with submicron sized Fe_2O_3 particles) that was outside the category boundaries. Seven *in vivo* studies were excluded because the test materials studied were not relevant to the category (*e.g.*, silica, carbon black, and diesel exhaust particulates).

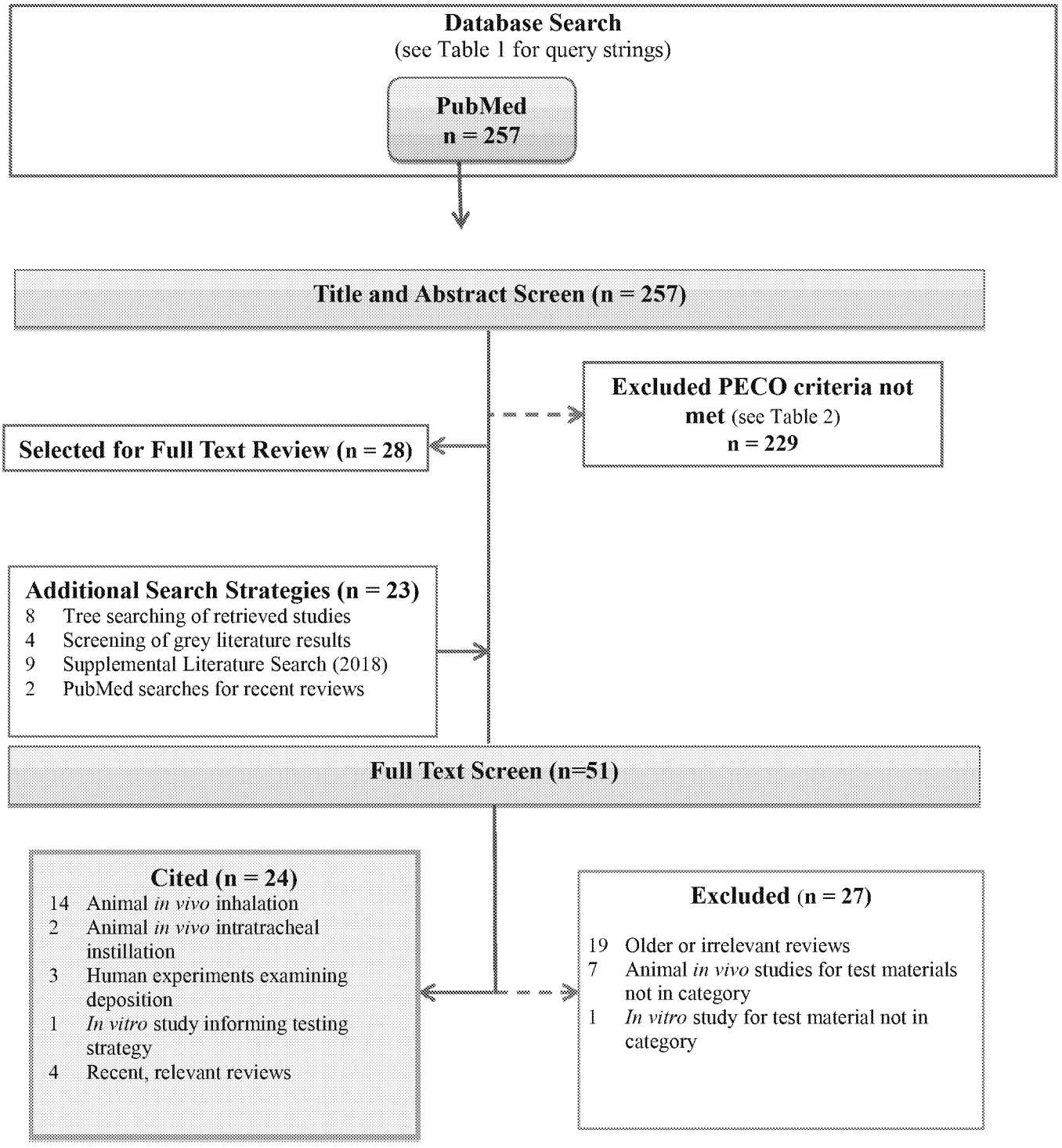

Figure [SEQ Figure * ARABIC]. Literature search and screening flow diagram for lung overload from HMW polymers.

B. Supplemental Literature Search

i. Search Strategy

To identify hazard concerns associated with inhalation exposure to poorly soluble polymers that would be in the category of polymer lung overload, the search strings presented in [REF _Ref46547800 \h * MERGEFORMAT] and [REF _Ref46547863 \h * MERGEFORMAT] were used for PubMed and Embase, respectively, to be more comprehensive. The results for this review are presented in [REF _Ref46548065 \h * MERGEFORMAT].

Table [SEQ Table * ARABIC]. PubMed Search strategy for polymer lung overload.

(((Aerosols[mh] OR polymers[mh] OR polyacrylate* OR methacrylate OR methacrylate[mh] OR polyvinyls OR polyvinyls[mh] OR "polyvinyl chloride"[mh] OR polystyrenes[mh] OR (toner AND (plastic OR printer OR powder OR xenograph*))) AND ("particulate matter"[mh] OR "particulate matter"[tw] OR dust[mh] OR dust OR particulate OR particle OR respirable OR insoluble OR "high molecular weight") AND (overload[tw] OR Lung diseases/chemically induced[mh] OR "pulmonary toxicity" OR "pulmonary function test" OR "pulmonary function tests" OR "respiratory function tests"[mh] OR "bronchoalveolar lavage fluid"[mh] OR "alveolar macrophage-mediated clearance" OR (lung[mh] AND (burden OR retention OR clearance OR absorption[mh] OR inflammation OR inflammation[mh] OR fibrosis OR fibrosis[mh] OR neoplasms OR neoplasms[mh] OR "cell proliferation"[mh] OR weight OR histopathology OR irritants[mh] OR irritancy OR irritation))) AND (((exposure OR administration) AND (intratracheal OR intranasal OR inhalation*)) OR "inhalation exposure"[mh] OR "in vitro" OR "in silico"))) OR (lung[tw] AND particle[tw] AND overload[tw]) NOT (iron[mh] OR calcium[mh] OR heart[mh] OR cardiac[tw] OR copper[mh] OR wildfire) AND English[lang]

Table [SEQ Table * ARABIC]. Embase Search strategy for polymer lung overload.

(lung AND particle AND overload OR (('aerosol'/exp OR 'polymer'/exp OR polyacrylate* OR methacrylate OR 'methacrylic acid'/de OR polyvinyls OR 'polyvinylchloride'/exp OR (toner AND (plastic OR printer OR powder OR xenograph*))) AND ('particulate matter'/exp OR 'particulate matter' OR 'dust'/exp OR dust OR particulate OR particle OR respirable OR insoluble OR 'high molecular weight') AND (overload OR 'lung disease'/exp OR 'pulmonary toxicity' OR 'pulmonary function test' OR 'pulmonary function tests' OR 'lung function test'/exp OR 'bronchoalveolar lavage fluid'/exp OR 'alveolar macrophage-mediated clearance' OR ('lung'/exp AND (burden OR retention OR clearance OR absorption OR inflammation OR 'inflammation'/exp OR fibrosis OR 'lung fibrosis'/exp OR neoplasms OR 'neoplasm'/exp OR 'cell proliferation'/exp OR weight OR histopathology OR 'irritant agent'/de OR irritancy OR irritation))) AND ('in vitro' OR 'ex vivo' OR 'in silico' OR 'inhalation'/exp OR ((exposure OR administration) AND (intratracheal OR intranasal OR inhalation*))))) AND [embase]/lim NOT ([embase]/lim AND [medline]/lim) AND 'article'/it AND [english]/lim

ii. Study question and PECO criteria

The study objective was to identify properties of particles that fall into the polymer lung overload chemical category and cause lung toxicity from particle overload and impaired clearance following inhalation exposure. The study question was:

> What are the physical-chemical properties of insoluble, high-molecular-weight polymer particles that result in particle overload corresponding to measures of lung toxicity (*i.e.*, chronic inflammation, cell proliferation) following exposure *via* inhalation?

A study reported in the peer-reviewed literature was determined to be relevant and selected for full-text review, or excluded, based on the PECO criteria outlined in **[REF _Ref46548160 \h * MERGEFORMAT]**, in which study populations, study design, comparison groups, and measured outcomes are identified. Studies identified for full-text review were not scored for quality, but were reviewed with quality in mind to provide critical information that supports the relationship between decreased particle clearance, particle overload, and lung effects (*i.e.*, chronic inflammation, cell proliferation, fibrosis, *etc.*), which is the proposed mode of action for this category. Exposure levels at which toxicity occurs, along with responses that may be influenced by factors such as particle

characteristics were indicated as relevant information for capture to address the study question. Included in this assessment were other routes of administration that have been used to evaluate particle overload in animal models, such as intratracheal and intranasal administration.

Table [SEQ Table * ARABIC]. PECO criteria for polymer lung overload.

Population	Humans and animal models that characterize lung polymer particle clearance kinetics and toxicity, or *in vitro* study models that inform lung toxicity related to kinetics or toxicity Exclude: unhealthy human populations; disease-induced experimental animals
Exposure	Inhalation exposure (including intratracheal and intranasal administrations) to particles that are classified as insoluble, high-molecular-weight polymers
Comparator	No particle exposure (*i.e.*, room air or no exposure), vehicle control (including intratracheal and intranasal administration and *in vitro* studies)
Outcome	Properties of polymer particles, lung particle overload, polymer particle clearance kinetics, or lung toxicity

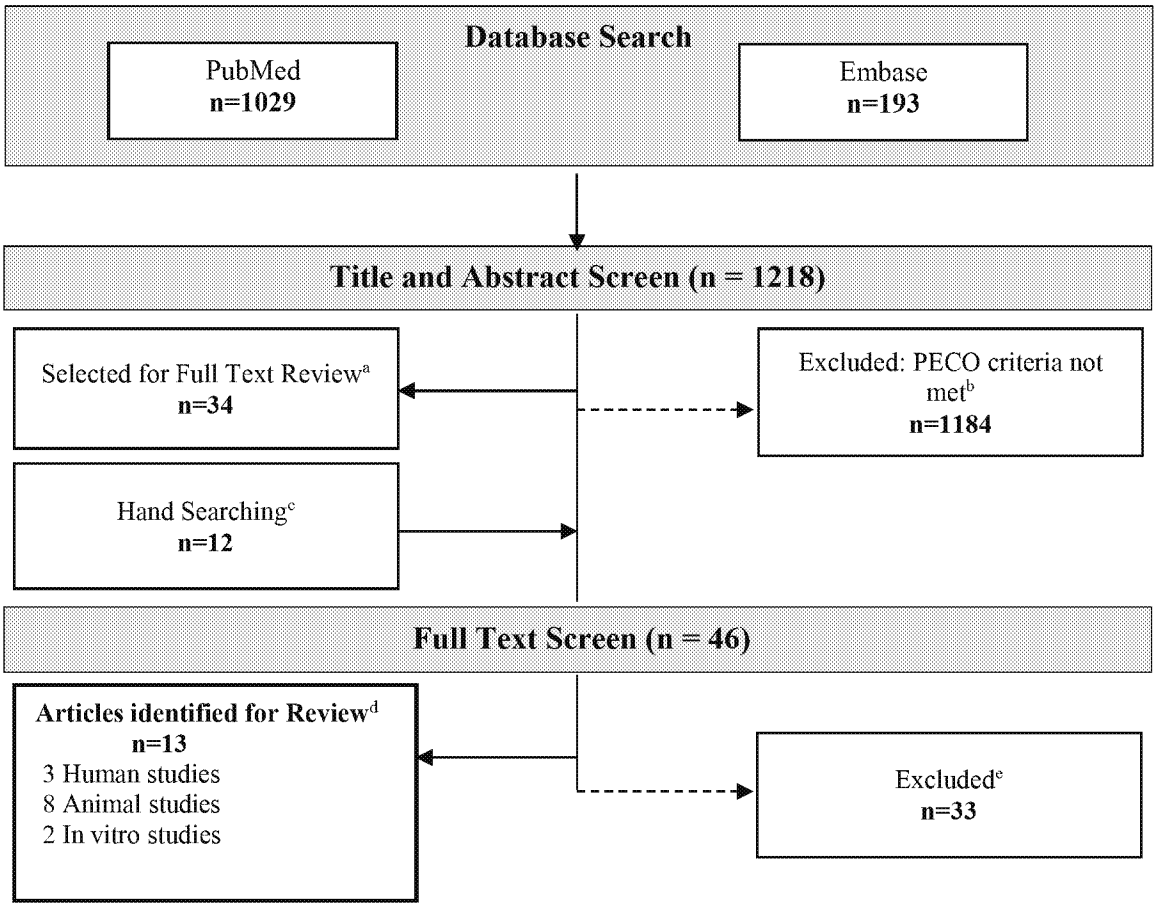

Figure [SEQ Figure * ARABIC]. Polymer lung overload: search strategy and results. [a] Selected based on title and abstract screen; [b] Excluded based on title and abstract screen; [c] Identified by hand-searching, either found in articles reviewed, or identified in the Initial Literature Search; [d] Studies identified as relevant for integrating into hazard summary; and [e] Key studies and review articles saved and used for contextual information are separately in the reference list.

iii. Hazard concerns

The hazard concerns associated with exposure to polymer lung overload particles are limited to effects on the lungs as a result of inhaling the particles. The substance may overload clearance mechanisms of the lung/respiratory system, resulting in effects that range from inflammation to fibrosis of the lungs often lead to altered lung function. There is a concern that the induction of chronic inflammation and fibrosis with chronic exposure, would result in lung cancer. Although carcinogenic effects have been demonstrated for poorly soluble inorganic particles, this has not been the case for the poorly soluble polymers defined in this chemical category. In a workshop hosted by ILSI and published in 2000 (ILSI, 2000), it was stated, "Because it is still not known with certainty whether high lung burdens of poorly soluble particulates can lead to lung cancer in humans via mechanisms similar to those of the rat, in the absence of mechanistic data to the contrary it must be assumed that the rat model can identify potential carcinogenic hazards to humans."

The review by ILSI (2000) stated that in the rat model, responsiveness to overload is associated with chronic inflammation and cell proliferation; therefore, when particle dose levels occur where these measures of response are not increased, it is anticipated that exposure would not result in lung tumors. This particle overload mode of action includes key events such as decreased lung particle clearance, retained particle burden in the lung that

exceeds a certain threshold, impairment of alveolar macrophage (AM) clearance function, AM accumulation, pulmonary inflammation, alveolar epithelia hyperplasia (proliferation), metaplasia, fibrosis, and possibly induction of lung tumors (reviewed by Borm et al., 2015; Warheit et al., 2016). A very thorough review of the pathobiology of lung overload is also presented in an ECETOC technical report (2013) which outlines the impact of biosolubility of the particles on their ability to be cleared. In short, poorly soluble particles that are inhaled are removed mainly by AM clearance and not by dissolution.

At the center of the particle overload hypothesis is the question about the human relevance of both non-neoplastic and neoplastic effects observed specifically in rats chronically exposed to high concentrations of poorly soluble particles of low acute toxicity (Borm et al., 2015). In the more recent review by Warheit et al. (2016), critical insights on differences in pulmonary response between rat exposure studies and occupationally exposed humans are identified. Although this review focuses on particle overload with inorganic particulates such as TiO_2, the species differences for particle deposition and overload for identification of hazard concerns for these and polymer lung overload particles, as defined in this category, are the same. These factors are critical considering that, at this time, it is still proposed that rat studies be used to evaluate hazard concerns for occupationally exposed workers. Considerations for evaluating lung effects from exposure to polymer lung overload in rats for extrapolation to humans include:

- Interspecies differences in lung responses of rats versus other rodents
- Interspecies differences in inhaled particle kinetics in rats versus nonhuman primates and humans that trigger particle-related responses in the lung
- Critical advancements in understanding the human respiratory tract and models of deposition and retention; used for simulating realistic particle translocation and retention
- Morphological differences and characteristics of rats for human lung tumor types and locations in the respiratory tract
- Epidemiological data from production works that demonstrate no correlation between particle exposure and lung cancer or other non-malignant respiratory diseases

Consideration of these differences between rodents and humans is at the core of this Supplemental Literature Review, especially when recommendation of further testing in the rat model is proposed. A tabular summary of peer-reviewed publications identified for full-text review is provided in [REF _Ref46548287 \h * MERGEFORMAT]. Study summaries with respect to the PECO criteria identified in this Supplemental Literature Review are provided below by study category (*i.e.*, human, animal *in vivo,* and *in vitro*) and are summarized with general comparisons to the findings presented in the Initial Literature Search. Critical information was documented that drove data collection for the development of a database of identified parameters associated with the hazards of concern for the polymer lung overload chemical category.

Given the limited information available in the literature for both human and *in vitro* studies on polymer lung overload particles as defined, selected particles that would not necessarily be included in this chemical category were considered for the Supplemental Literature Review, as the identified studies may be relevant concerning particle clearance kinetics or potential *in vitro* assay systems for consideration in an alternative non-animal testing strategy.

Table [SEQ Table * ARABIC]. Peer-reviewed publications identified for full-text review.

Author/Title	Defined test substance	Particle characteristics (physical form, Molecular weight, *etc.*)	Study type / Model	Exposure route / concentrations	Study description	Aerosol / particle size	Outcomes / Toxicity	Authors' conclusions
Bai et al., 2010. Pulmonary responses to printer toner particles in mice after intratracheal instillation.	Toner; collecting during a printing process $PM_{2.5}$ and PM_{10}	N/A	*In vivo* / male ICR mice	Intratracheally instillation/40 mg/kg	Instillation of dose formulation was performed 4 times (every 2 days). Toxicity evaluation was conducted at 9, 28, 56, and 84 days post instillation. Control group - no instillation. The pulmonary responses were measured bronchoalveolar lavage fluid (BALF) for biochemical analysis and lung tissue histopathology and electron microscopy.	The toner particles administered to the animals were not clearly defined, assumed to be $PM_{2.5}$ and PM_{10}	It was noted that during the experimentation period toner particles were shown to adhere to the alveolar septal walls and enter into the alveoli to cause pulmonary lesions. Lung overloaded by toner particles caused an inflammatory response and damaged alveolar epithelial -capillary barrier and increased cell permeability. Although this type of administration does not provide realistic exposure and lung burden/clearance data, it does confirm that damage that occurs where particles are administered to the lung.	Authors state "The results of biochemical analysis of BALF and lung homogenates indicated that the lung were overloaded by toner particles, which induced inflammatory response, damaged alveolar epithelial-capillary barrier and increased cell permeability. The increased phagocytosis of particles by AMs in lungs was observed over time. The normal lung structure was damaged simultaneously. In this study, we did not find pulmonary fibrosis or mesothelioma formation throughout the experiment."
Bellman et al., 1991. Lung clearance and retention of toner, utilizing a tracer technique, during chronic inhalation exposure in rats. Identified in Initial Literature Search.	Only identified as "Special Test Toner"	N/A	*In vivo*, male and female F344 rats	Inhalation exposure, 0, 1, 4, 16 mg/m^3 - toner and TiO_2 at 5 mg/m^3 or SiO_2 at 1 mg/m^3.	Rats exposed *via* exposed 6 hr/day, 5 days/week up to 24 months - pulmonary retention measured after 3, 9, 15, 21 and 24 months	Mass median aerodynamic diameter (MMAD) was geometric standard deviation of not identified	The final pulmonary burdens of toner at the 3 exposure levels were 0.22, 1.73, and 15.6 mg/lung. Clearance was impaired at the mid- and high exposure level. Both the maximum tolerated dose and the maximum functionally tolerated dose were exceeded at the toner high exposure level during the study in rats. MTD and MFTD is based on clearance - Boundaries for safety would have to be determined to ensure	As noted by the authors, alveolar clearance of toner was impaired at the high, and moderately slowed at the mid exposure levels. At high-exposure it was associated with lung overloading. For inhalation of insoluble particles Muhle et al. 1990 suggested the use of maximum functionally tolerated dose (MFTD) concept, in terms of a lung burden at which macrophage-mediated clearance half-time is increased by a factor of 2-4.

							exposure levels over a period of time would be able to be cleared, etc. Note: Based on the lack of toner particle information and the lack of response data- this article was extracted but does not provide all necessary information for an evaluation.	
Bellman et al., 1992. Irreversible pulmonary changes induced in rat lung by dust overload. Identified in Initial Literature Search.	9000-type xerographic toner material composed of 90% styrene/1-butylmethacrylate random copolmer-with 10% high purity furnace type carbon black [CAS 25213-39-2/ 7440- 44-0]	N/A	*In vivo* / female F-344 rats	Inhalation exposure, 0, 10, 40 mg/m^3; methods described by Muhle et al., 1991	Rats exposed via nose-only inhalation 6 hrs/day, 5 days/wk, for 3 months at 4 doses (plus control). Recovery groups (4 wks recovery) were included in control and high dose groups. Clinical chemistry, tissue weights and histopathology were examined. Tracer aerosols were inhaled (NO) for 0.5-1.0 hr by eight animals for analysis of lung burden. Retention of test toner in the lungs and in the lung-associated lymph nodes was analyzed in lung tissue. BALF (cytology and cell toxicity measures, LDH, β-glucuronidase and total protein). The distribution of test toner particles in macrophages was analyzed by light microscopy. Lung tissue evaluated for histopathological changes. This study was conducted to investigate if toxicity associated with overload would be reversed following cessation of exposure and clearance of particles.	Mass median aerodynamic diameter (MMAD) was 4.0 µm with a geometric standard deviation of 1.5	Overall half-times of toner clearance were calculated as 277 and 2845 days at 10 and 40 mg/m^3, respectively. The retained test toner at the end of exposure was 0.4 and 3.0 mg in the lung for the low and high exposure groups, respectively. At the high exposure, LDH, β-glucuronidase and total protein were elevated with a minor recovery after 15 months. Differential cell count was only slightly increased in the number of PMN with significant response at the high concentrations. Alveolar clearance was delayed at the low exposure, but most completely impaired at the high exposure level with significance after 3 months.	As noted by the authors, in the high exposure group, the pattern of the effects during the 15 - month post-treatment observation period was similar (based on measures of toxicity). Much of these study findings were based on high exposure. It is critical to note that the 10 mg/m^3 exposure did not show changes in LDH, β-glucuronidase, and total protein

Klas et al., 2009. Does lung retention of inhaled particles depend on their geometric diameter?	^{111}In - Labeled polystyrene and Teflon particles	Densities of particles were 1.05 g/cm^3 for polystyrene and 2.13 g/cm^3 for Teflon.	Humans, 9 healthy nonsmokers	Length of inhalation exposure varied between 3- 5 minutes and number of inhalations between 4 and 6. Flow of 0.045 L/s. Note: focus on ciliary airways; Lung deposition was modeled.	Particle inhalation followed by radioactivity distribution at 24, 48, and 72 hours post exposure using NaI crystals fitted with collimators and profile scanning over the mouth, throat, lungs and stomach of each subject. Theoretical calculations of lung deposition were	Mean aerodynamic diameter is 6.2 and 6.5 µm for polystyrene and Teflon particles, respectively with the mean geometric diameter was 6.05 µm and 4.47 µm for the polystyrene and Teflon particles, respectively with the geometric standard deviation 1.06 for both.	Particle deposition in the lung averaged 20 and 68 (lung retention at 24 hours as a percental of initial lung deposition) for polystyrene and Teflon, respectively.	This study did not show evidence that the fraction of particles deposited in the conducting airways and their retention is dependent on geometric diameter in the size range studied. Note: when exposure is low particle overload that occurs in rat models cannot be evaluate in human studies.
Konczol et al., 2013. Oxidative stress and inflammatory response to preinter toner particles in human epithelial A549 lung cells	Printer toner powders Carbon-bearing particles (2–12 µm), rough surface covered with Fe_3O_4 sub-micron particles (30–200 nm); Presence of rutile, cristobalite, perovskite	N/A	*In vitro*, human epithelial A549 lung cells; human lung adenocarcinoma type-II alveolar epithelial cells	Particle suspensions in RPMI 1640 supplemented with 1% L-glutamine and 1% penicillin/streptomycin, 2.5 µL tween 20 for homogeneous dispersion	Cells were exposed to. Toner at 20-200 µg/cm^2 / 0 µg/cm^2 for up to 24 hours.	2–12 µm, particles 30–200 nm	Endpoints included mitochondrial membrane depolarization, NF-kB binding activity, IL-6 and IL-8 levels along with measures of cytotoxicity using water-soluble tetrazolium assay and neutral red uptake.	Overall exposure to these toner particle suspensions resulted in a concentration dependent formation of reactive oxygen species and measures of oxidative stress through induction of reactive oxygen species that could result in activation of pro-inflammatory pathways.
Lee et al., 1988. Lung response to ultrafine Kevlar aramid synthetic fibrils following 2-year inhalation exposure in rats.	Kevlar fibrils (<3.0 um in diameter) and < 100 µm in length	N/A	*In vivo*, male and female Crl:CD (SD)BR rats	Exposed by inhalation to ultrafine Kevalr fibrils at 2.5, 25 or 100 fibrils/cc	Rats were exposed for 6 hr/day, 5 days/week for 2 years. Lung response to ultrafine Kevlar synthetic fibrils following 2-year inhalation exposure in rats	Exposure to Kevlar fibers at 2.5 to 400 fibers/cc with a mean mass concentration of 0.08 to 2.23 mg/m^3. The fibril/mass ratio was 30 to 184.	Kevlar exposed rats did not develop mesothelioma or different histological types of lung tumors including papilloma, bronchioloalveolar adenoma, bronchogenic carcinoma, squamous cell carcinoma, fibrosarcoma, or adenocarcinoma. No pathological lesions attributable to Kevlar dust exposure in lungs, without	

							any observation of hyperplastic epithelial change nor inflammatory response to deposition. It is noted that the majority of Kevlar fibrils were phagocytized by single macrophages in the alveoli adjacent to the alveolar duct region. Note: particle deposition was confined to the alveolar duct region, particle overload was not identified by name, but this is an early publication. There is no evidence in this study that fiber overload was demonstrated with decreased clearance.	
Muhle et al., 1990a. Subchronic inhalation study of toner in Rats. Identified in Initial Literature Search.	9000-type xenographic toner material composed of 90% styrene/1-butylmethacrylate random co-polymer- with 10% high purity furnace type carbon black. The polymer was composed of styrene and 1-butylmethacrylate ratio of 58:42 [CAS no. 25213-39-1/ 7440-44-0]	MW 70,000 Daltons	*In vivo*, female F344 rats	Whole body, inhalation exposure/ 0, 4, 16, 64 mg/m^3 - corresponded to 0, 0.35, 1.4, 5.6, 22.4 mg/m^3 of respirable material	6 hr/day, 5 days/week for up to 13 weeks. Retention and clearance measurements after 30, 60 and 90 days at 1 and 64 mg/m^3 and after 45 and 90 days.	Mass median aerodynamic diameter (MMAD) was 4.0 µm with a geometric standard deviation of 1.5,	Lung weight and histopathology, respiratory volume and frequency, lung burden of toner, deposition rate, alveolar clearance and retention. Both wet and dry weights of left lung were elevated at highest concentration, with deposition of toner 5.8-fold higher at 64 v. 16 mg/m^3. Based on retention of toner, there was a greater than proportional increase retained at 16 and 64 mg/m^3 suggesting overloading. Clearance mechanisms were impaired at high exposures up to 90 days. Lungs had increased particle - laden macrophages with few in alveolar walls.	

Muhle et al., 1990b. Dust overloading of lungs after exposure of rats to particles of low solubility: Comparative studies. Identified in Initial Literature Search.	Test toner (carbon black pigmented acrylic polymer), polyvinyl chloride powder, carbon black (type 'Printex 90', and two modifications of TiO^2	N/A	*In vivo*, rats (limited reporting)	Exposure not described with concentrations reported as Toner 1, 4, 16.1, 63.2 mg/m^3, PVC 3.3, 8.3, 20.2 mg/m^3, Carbon black, 9 mg/m^3 compared to control 0 mg/m^3	Exposure for up to 2 years at selected time points.	Diameter (µm), MMAD (µm) geometric SD, density: Toner ~ 4, 4, 1.5, 1.15; PVC ~ 1.3, 1.3, 2.07, 1.3; Carbon black ~0.014, 0.96, 2, 2	Particle clearance, measures of cytology (BALF) lung histology. Exposure resulted in retardation of alveolar clearance when retained mass reached a level of 0.5 mg per rat lung with over 700 days of overloading (>10 mg per rat lung. After 24 months, an increase in PMN (polymorphonuclear cells) was observed at 4 and 16 mg/m^3.	"Characteristic findings of dust overloading of lungs are: (a) alveolar clearance retardation, (b) increased retention of material in the lung, (c) increase in lung weight, (d) accumulation of dust laden macrophages, (e) persistent inflammation, (f) increased epithelial permeability, and (g) elevated infiltration of neutrophils. Specific histopathological findings will be presented elsewhere."
Muhle et al., 1991. Pulmonary response to toner upon chronic inhalation exposure in rat s. Identified in Initial Literature Search.	Test toner [contained 90% random copolymer CAS No. 25213-39-2) and 10% high purity carbon black CAS No. 7440-44-0]; polymer composed of styrene and 1-butyl methacrylate (58:42)	Density 1.2 g/cm^3, MW 70,000 Daltons	*In vivo*, F344 male and female rats	Whole body inhalation exposure using a dry aerosol dispersion technique. Target concentrations 0, 1, 4, 16 mg/m^3 of toner.	Rats were exposed 6 hr/day, 5 days/wk for up to 24 months	MMAD ~ 4.0 µm with GSD of 1.5	BALF (cytology, LDH, β-glucuronidase, total protein), lung histopathology and lung were measured. Animals were reported to not show any clinical signs and appeared healthy at the end of exposure. Both absolute and relative lung weights were increased with increased retention of particles. Elevation in measured in BALF were observed with increased toner concentration. There was an exposure and time-dependent increase in the extent of particle-laden macrophages in lungs of toner-exposed rats. Toner increased primary lung tumors from 1 (adenoma) in the low dose to 3 tumors at the high concentration, without tumors identified in the mid concentration.	Author states "The appropriate manner to compare various dusts is by density-corrected mass or volume of material tin the lungs."

Oberdorster et al.; 1992. Volumetric loading of alveolar macrophages (AM): A possible bassi for diminished AM- mediated particle clearance. Identified in Initial Literature Search.	^{141}Ce-labeled 3.3 µm diameter and ^{95}NB-labeled 10.3 µm diameter polystyrene microspheres were obtained as dry particles.	N/A	*In vivo*, male Fischer 344 rats	Intratracheally instillation of polystyrene particles; 40 µg and 100 µg of 3.3 µm particles and 10 and 100 µg of 10.3 µm particles - mixture of ^{141}Ce and ^{95}NB-polystrene particles - Note: although useful in understand the mode of action, the exposure following intratracheally administration is different from inhalation exposure.	Animals were administered particles by intratracheal instillation and killed to evaluate lung retention, cytology measurements in lung lavage for particle recovery (did not work well) and histopathology at 132- and 202-days post administration.	Diameter 3.3 µm and10.3 µm; converting administered mass to volume; low dose received 2.2E7 µm^3 and the high dose group received ~1.1E8 µm^3.	Overall particle clearance was AM (alveolar macrophage) mediated with translocation through the tracheobronchial tree in the GI. The particle associated radioactivity in the BL was the same whether it was large or small particles. There was no histological evidence for particle sequestration suggested by the aggregation of AM.	Authors state "We conclude from these studies in the Fischer 344 rat that (1) large (10.3 µm) particles are readily phagocytized by AM, (2) phagocytized 10.3-pm particles significantly reduce AM clearance function, (3) pulmonary clearance of 3.3 µm and 10.3 µm particles at lung burdens of 100 µg/rat lung occurs *via* the tracheobronchial tree into the GI tract, and (4) our results support the volumetric hypothesis of retardation of AM-mediated particle clearance."
Smith et al., 2008. Effect of particle size on slow particle clearance from the bronchial tree	Nontoxic insoluble particles; ^{99}mTC and ^{111}In labeled polystyrene latex (PSL) and ^{198}Au labeled gold	PSL: density 1.05 g/cm^3; gold: density 19.3 g/cm^3	Healthy volunteers, non- or stopped smokers.	Particles were administered as a bolus of an aerosol into a specific depth of the lungs using a nebulizer, monitoring breathing and lung capacity, to obtain the required initial lung deposit of particles.		Aerodynamic diameter of 5 µm, Gold particles geometric diameter 1-2 µm with aerodynamic diameter of 4.9 µm	Study was designed to test the parameter of particle geometric diameter on clearance of particles form the bronchial tree. Although, clearance from the bronchial tree is not the mode of action of particle overload, information on particle diameters was of interest. In this study there was no difference in the retention of 1 µm gold particles and 5 µm polystyrene particles.	

Svartengren et al., 2001. Comparison of clearance of particles inhaled with bolus and extremely slow inhalation techniques.	Teflon particles labelled with ^{111}In	N/A	Human, 10 healthy nonsmoker volunteers	Volunteers inhaled Teflon particles labelled with ^{111}IN with a shallow bolus technique and an extremely slow (~0.05L/s) inhalation technique. There was an interval between the 2 inhalation exposures by 1 month	Following inhalations of either method, radioactivity in the lungs was measured at 1 and 24 hours, and then 1 2, and 3 weeks post exposure.	Geometric particle diameter was 4.4 µm with the GSD 1.05 for the bolus and 1.07 for the slow inhalation. MMAD was 6.4 µm	Forced vital capacity (FVC) Forced expiratory volume in 1 s (FEV1,0) and forced expiratory flow between 25 and 75% (FEF25-75%) of the exhaled volume was only used to compare subjects and parameters for modeling. Using a scan to follow deposition of radiolabeled particles and distribution in the lung was monitored. Bolus v. slow inhalation did not appear to change the lung deposition of these particles. Although clearance evaluated, the deposition of these particles was not in the alveolar space or appeared to be at the concentration where lung overload conditions, similar to what would occur in rats could be evaluated.	Bolus v. slow inhalation did not appear to change the deposition or extent of the particles to various lung regions.
Wiemann et al, 2016. An in vitro alveolar macrophage assay for predicting the short- term inhalaiton toxicity of nanomaterials. Identified in Initial Literature Search.	18 inorganic nanomaterials, covering AlOOH, $BaSO_4$, CeO_2, Fe_2O_3, TiO_2, ZrO_2, and ZnO NMs, amorphous SiO_2 and graphite nanoplatelets, and two nanosized organic pigments. ZrO_2 and amorphous SiO_2 were tested without and with surface functionalization. ***NOTE:*** This study does not meet the defined chemical category but was identified as a possible	N/A	*In vitro*, rat NR8383 alveolar macrophage s	Test materials were incubated with cells in protein free culture medium	LDH, glucuronidase and tumor necrosis factor alpha, and ROS/H_2O_2 were used to monitor responses after 16 hours of exposure.	Size (nM), surface areas BET (m^2/g), size in buffer, (nm) and concentration in buffer; ranged from 2 nm to < 30 µm, 15 to 200 m^2/g, not detectable to 290 nm, not detectable to 1.3E8 particles/mL, respectively	Based on particle surface area threshold, there were low in vitro effects compared to materials above the threshold (6000 mm^2/mL) that result in overloading and were considered active (2 of the 4 toxicity parameters). Overall this assay was highly predictable of short-term rat inhalation hazard potential.	Authors state "When integrated into a tiered testing approach, such as the DF4nanoGrouping, the in vitro NR8383 AM assay may substantially reduce the need for animal testing addressing the inhalation route of exposure. Further work should aim at validating this assay."

	method to evaluate insoluble particles *in vitro*.							

iv. Studies in humans

The Initial Literature Search did not identify human studies that describe exposure to the particles defined in this polymer lung overload category. In the Supplemental Literature Search, several studies were identified in humans that did not necessarily fulfil all the PECO criteria ([REF _Ref46548160 \h * MERGEFORMAT]) but provided information on particle parameters and lung clearance in humans. The identified studies are summarized in [REF _Ref46548446 \h * MERGEFORMAT] according to these PECO criteria and used to highlight critical information and/or gaps in the knowledge base ([REF _Ref46548287 \h * MERGEFORMAT]).

The studies in humans, outlined in [REF _Ref46548287 \h * MERGEFORMAT], note that parameters of particle geometric diameter and density do not seem to play a role in lung clearance. It is important to note that conditions of particle overload in AM were not evaluated, as lung deposition was in the bronchial tree, where clearance mechanisms are different (Svartengren et al., 2001; Smith et al., 2008; Klas et al., 2009). As is obvious, particle lung overload experiments could not be conducted ethically in humans, because the mechanism of lung overload would be through high chronic exposure conditions. The more recent review by Warheit et al. (2016) references an epidemiological investigation of exposure to toner, in which an absence of lung cancer excess risk due to dust exposures was reported. This study was not captured in the Initial Literature Search or the Supplemental Literature Search, though its review may be of use for strengthening the discussion that these particles do not presence a cancer hazard.

Table [SEQ Table * ARABIC]. Population: Human studies on polymer lung overload.

Reference	Polymer lung overload Particles	Exposure/Comparator	Clinical Outcomes/Toxicities
Klas et al., 2009.	Radiolabeled polystyrene particles and ^{111}In-labeled Teflon particles	Length of inhalation exposure was 3–5 mins, between 4 and 5 inhalations at a flow of 0.045 L/s. Particle deposition in the lung averaged 20 and 68 (lung retention at 24 hours as a percentage of initial lung deposition) for polystyrene and Teflon, respectively. Note: exposure and comparator information not clearly identified with focus on ciliary airways.	This study did not show evidence that the fraction of particles deposited in the conducting airways, and their retention, depend on geometric diameter in the size range studied. Note: when exposure is low, particle overload that occurs in rat models cannot be evaluated in human studies.
Smith et al., 2008.	Nontoxic insoluble particles; ^{99}mTC and ^{111}In labeled polystyrene latex (PSL) and ^{198}Au labeled gold; Densities PSL: 1.05 g/cm^3, Gold: 19.3 g/cm^3	Particles administered as a bolus of an aerosol into a specific depth of the lungs using a nebulizer, monitoring breathing and lung capacity, to obtain the required initial lung deposit of particles. Gold: 1μm particles PSL: 5 μm particles	Study was designed to test particle geometric diameter on particle clearance from the bronchial tree. Although clearance from the bronchial tree is not the mode of action of particle overload, information on particle diameter and density was of interest. In this study, there was no difference in the retention of gold and PSL, particles of different densities and diameters.

Svatengren et al., 2001.	^{111}In-labeled Teflon particles	Volunteers inhaled ^{111}In labeled Teflon particles with a shallow bolus technique and an extremely slow (~0.05 L/s) inhalation technique; 1-month interval between two exposures, with lung radioactivity measured at 1 and 24 hours, and then at 1, 2, and 3 weeks post-exposure. Geometric particle diameter was 4.4 µm with the geometric standard deviation (GSD) 1.05 for the bolus and 1.07 for the slow inhalation. Mass median aerodynamic diameter (MMAD) was 6.4 µm.	Bolus v. slow inhalation did not appear to change the lung deposition of these particles. Although clearance was evaluated, these particles were not deposited in the alveolar space and appeared to be at the concentration where lung overload conditions, similar to what would occur in rats, could be evaluated.

v. Studies in animal and in vitro models

In vivo animal and *in vitro* studies are summarized in [REF _Ref46548546 \h * MERGEFORMAT] and [REF _Ref46548653 \h * MERGEFORMAT], respectively, according to the PECO criteria that were used to identify critical information and/or gaps in the knowledge base ([REF _Ref46548287 \h * MERGEFORMAT]).

In agreement with the findings from the Initial Literature Search, only limited information was identified in the Supplemental Literature Search which evaluated particles that induce adverse lung effects through overload and saturation of clearance mechanisms ([REF _Ref46548546 \h * MERGEFORMAT]). In summary, the older studies identified in the Initial Literature Search (Bellman et al., 1991, 1992; Muhle et al., 1990a,b, 1991) may have deficiencies in either study design and/or reporting, but they capture the dynamics of these particles in the lung and provide a foundation for establishing that the particle overload mode of action is driving the adverse effects following inhalation exposure to polymer lung overload particles.

In the search strategy employed in the Supplemental Literature Search, many of the same articles were identified from the Initial Literature Search, even though the search strategy was expanded to include other routes of administration, such as intratracheal instillation, in an effort to capture more data on particle characteristics, AM overload, and decreased clearance. One additional study was identified that met the PECO criteria in which rats were administered the test substance by intratracheal instillation (Bai et al., 2010). In comparison, one study was identified in the Initial Literature Search that evaluated polymer lung overload from a substance following intratracheal instillation (Oberdorster et al., 1992). There are a number of studies in the literature that administered particles via intratracheal instillation; however, these were not particles identified in the Initial Literature Search as within the polymer lung overload category. The study conducted by Oberdorster et al. (1992) supported that intratracheal instillation could be used to study the mode of action of particle lung overload induced lung toxicity. Although biosolubility of a particle has been identified as being critical to the particle overload mode of action, no information presented in the studies reviewed provided this type of information for the polymer lung overload particles tested.

Only two *in vitro* assays were identified in the Supplemental Literature Search with potentially useful information- one of which was originally identified in the Initial Literature Search (Wieman et al., 2016). Although Wieman et al. (2016) did not test particles that would meet the polymer lung overload category, the significance of this assay is that rat NR8383 alveolar macrophages were used, and therefore, a measure of particle clearance kinetics and toxicity could be evaluated. This assay was highly predictive of short-term rat inhalation hazard potential for inorganic particulates. The other *in vitro* assay identified in the Supplemental Literature Search (Konczol et al., 2013) used human epithelial lung cells (A549), since it does not include AM, necessary to capture the particle

lung overload mode of action, it might not be useful to evaluate polymer lung overload particles in this chemical category.

Table [SEQ Table * ARABIC]. Population: Animal studies on polymer lung overload.

Reference	Polymer lung overload particles	Exposure /Comparator	Outcomes/Toxicities
Bai et al., 2010.	Toner; collected during a printing process, $PM_{2.5}$ and PM_{10}	Mice, intratracheal instillation 4 times (every 2 days) and evaluated up to 84 days post-exposure at 40 mg/kg saline or no instillation. The toner particles administered to the animals were not clearly defined, assumed to be $PM_{2.5}$ and PM_{10}	Exposure to toner particles resulted in a significant inflammatory response and lung lesions associated with an increase in alveolar macrophage numbers with increased apoptosis.
Bellman et al., 1992. Identified in the Initial Literature Search	9000-type xerographic toner material composed of 90% styrene/1- butylmethacrylate random copolmer- with 10% high-purity furnace-type carbon black [CAS 25213-39-2/ 7440-44-0]	Rats, 6 hr/day, 5 days/week for 3 months, Inhalation exposure to aerosol 10, 40 mg/m^3 / 0 ppm. Mass median aerodynamic diameter (MMAD) was 4.0 μm, with a geometric standard deviation of 1.5.	Toner clearance half-times increased with exposure, 277 and 2845 days at 10 and 40 mg/m^3, with 0.4 and 3.0 mg lung burden at end of exposure. LDH, β-glucuronidase, and total protein increases showed a minor recovery after 15 months at the high concentration. Differential cell count was significantly increased at the high concentration; number of polymorphonuclear cells. Alveolar clearance was delayed at the low, but almost completely impaired at the high concentration after 3 months.
Bellman et al., 1991. Identified in Initial Literature Search Note: Based on the lack of toner particle information and the lack of response data, this article was extracted but does not provide all necessary information for an evaluation.	Only identified as "Special Test Toner"	Rats, exposed by inhalation at 6 hr/day, 5 days/week up to 24 months, 1, 4, 16 mg/m^3 / 0 mg/m^3; the final pulmonary burdens of toner at the 3 exposure levels were 0.22, 1.73, and 15.6 mg/lung. MMAD or GSD not provided.	Particle retention was evaluated; both the maximum tolerated dose (MTD) and the maximum functionally tolerated dose (MFTD) were exceeded at high exposure level. MTD and MFTD are based on clearance documented within the context of Muhle et al., 1991.

Konczol et al., 2013.	Carbon-bearing particles (2–12 μm); rough surface covered with Fe_3O_4 submicron particles (30–200 nm). Presence of rutile, cristobalite, perovskite.	*In vitro*, human epithelial A549 lung cells exposed to particle suspensions in media for up to 24 hours. Cells exposed to 20–200 $\mu g/cm^2$ / 0 $\mu g/cm^2$.	Exposure to toner particle suspensions showed a concentration dependence in measures of oxidative stress through induction of reactive oxygen species that could result in activation of pro- inflammatory pathways. Not evaluated with respect to particle lung overload.
Lee et al., 1988.	Kevlar fibrils	Rats were exposed for 6 hr/day, 5 days/week for 2 years to ultrafine Kevlar fibrils at 2.5, 25, or 100 fibrils/cc. Fibers <3.0 μm diameter and <100 μm length.	Kevlar-exposed rats did not develop mesothelioma, different histological types of lung tumors, or non- neoplastic lesions (hyperplastic epithelial change, nor inflammatory response to deposition) associated with exposure. Note: particle deposition was confined to the alveolar duct region; particle overload was not identified by name (early publication). There is no evidence in this study that fiber overload was demonstrated with decreased clearance.
Muhle et al., 1990a. Identified in the Initial Literature Search	9000-type xenographic toner material composed of 90% styrene/1-butyl- methacrylate random co-polymer, with 10% high-purity furnace- type carbon black. The polymer was composed of styrene and 1-butyl- methacrylate ratio of 58:42 [CAS no. 25213-39-1/ 7440-44-0]	Rats, 6 hr/day, 5 days/week for 13 weeks. Inhalation exposure to aerosol 4, 16, or 64 mg/m3 / 0 ppm. Mass median aerodynamic diameter (MMAD) was 4.0 μm, with a geometric standard deviation (GSD) of 1.5.	Both wet and dry lung weight was elevated at 64 mg/m^3, with deposition of toner 5.8-fold higher at 64 vs 16 mg/m^3. Based on retention of toner, there was a greater than proportional increase retained at 16 and 64 mg/m^3, suggesting overloading. Clearance mechanisms were impaired at high exposures up to 90 days. Lungs had increased particle-laden macrophages with few in alveolar walls.
Muhle et al., 1990b. (report was limited) Identified in the Initial Literature Search	Test toner (carbon black pigmented acrylic polymer), polyvinyl chloride (PVC) powder, carbon black (type 'Printex 90')	Rats, inhalation exposure for up to 2 years, animals taken off study to evaluate at selected timepoints. Concentrations: Toner- 1, 4, 16.1, 63.2 mg/m^3; PVC, 3.3, 8.3, 20.2 mg/m^3 0 mg/m^3, carbon black, 9 mg/m^3, Diameter (μm), MMAD (μm) geometric SD, density: toner ~4, 4, 1.5, 1.15. PVC ~1.3, 1.3, 2.07, 1.3 carbon black ~0.014, 0.96, 2, 2	Particle clearance, measures of cytology (bronchoalveolar lavage fluid; BALF), lung histology; exposure resulted in retardation of alveolar clearance when retained mass reached a level of 0.5 mg per rat lung, with over 700 days of overloading (>10 mg per rat lung). After 24 months, an increase in polymorphonuclear cells was observed at 4 and 16 mg/m^3.
Muhle et al., 1991.	Test toner (contained 90% random copolymer CAS No.	Rats, whole-body inhalation exposure, 6	Both absolute and relative lung weights increased with increased retention of particles. Elevated toxicity measures in BALF were observed with increased

Identified in the Initial Literature Search	25213-39-2) and 10% high-purity carbon black CAS No. 7440- 44-0]; polymer composed of styrene and 1-butyl methacrylate (58:42)	hr/day, 5 days/wk for up to 24 months using a dry aerosol dispersion technique. Target concentrations 0, 1, 4, 16 mg/m^3 of toner, MMAD ~ 4.0 μm with GSD of 1.5	toner concentration. There was an exposure and time- dependent increase in the extent of particle-laden macrophages in lungs of toner-exposed rats. Toner increased primary lung tumors from 1 (adenoma) in the low dose to 3 tumors at the high concentration, without tumors identified in the mid concentration.
Oberdorster et al., 1992. Identified in the Initial Literature Search	^{141}Ce-labeled 3.3 μm diameter and 95 NB-labeled 10.3 μm diameter polystyrene microspheres were obtained as dry particles. Note: although useful in understanding the mode of action, the exposure following intratracheal administration is different from inhalation exposure.	Rats, intratracheal instillation of polystyrene particles; 40 μg and 100 μg of 3.3 μm particles and 10 and 100 μg of 10.3 μm particles—mixture of ^{141}Ce and ^{95}NB-polystrene particles-Diameter 3.3 and 10.3 μm; converting administered mass to volume; low-dose group received 2.2E7 μm^3, and the high-dose group received ~1.1E8 μm^3.	Overall particle clearance was AM (alveolar macrophage) mediated with translocation through the tracheobronchial (BL tree in the GI. The particle-associated radioactivity in the BL was the same whether it was large or small particles. There was no histological evidence for particle sequestration suggested by the aggregation of AM.

Table [SEQ Table * ARABIC]. Population: *In vitro* studies on polymer lung overload.

Reference	Polymer lung overload particles	Exposure/Comparator	Outcomes/Toxicities
Konczol et al., 2013.	Carbon-bearing particles (2-12 µm), rough surface covered with Fe_3O_4 submicron particles (30-200 nm); presence of rutile, cristobalite, perovskite.	Human epithelial A549 lung cells exposed to toner at 20-200 µg/cm^2 / 0 µg/cm^2 for up to 24 hours.	Endpoints included mitochondrial membrane depolarization, NF-kB binding activity, IL-6 and IL-8 levels, along with measures of cytotoxicity using water-soluble tetrazolium assay and neutral red uptake.
Wiemann et al., 2016. Identified in the Initial Literature Search	18 Inorganic nanomaterials, covering AlOOH, $BaSO_4$, CeO_2, Fe_2O_3, TiO_2, ZrO_2, and ZnO NMs, amorphous SiO_2 and graphite nanoplatelets, and two nanosized organic pigments. ZrO_2 and amorphous SiO_2 were tested without and with surface functionalization.	Rat NR8383 alveolar macrophages exposed to 22.5 to 180 µg/mL / 0 µg/mL Nanoparticle size (nm), surface areas BET (m^2/g), size in buffer, (nm) and concentration in buffer; ranged from 2 nm to <30 µm, 15 to 200 m^2/g, not detectable to 290 nm, not detectable to 1.3E8 particles/mL, respectively.	LDH, glucuronidase and tumor necrosis factor alpha, ROS/H_2O_2 measures were used to monitor responses after 16 hours of exposure. Based on particle surface area threshold, there were low *in vitro* effects compared to materials above the threshold (6000 mm^2/mL) that result in overloading and were considered active (2 of the 4 toxicity parameters). Overall, this assay was highly predictive of toxicity reported in short- term rat inhalation hazard potential for the same test substances.

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2. Experimental Animal Inhalation Studies on HMW Polymers

Table [SEQ Table * ARABIC]. Inhalation studies of lung overload from HMW polymers in laboratory animals.

Test Material[a]	Animals	Exposure and Recovery	Lung effects	Reference
9000 Toner (styrene/butylmethacrylate random copolymer) MW: 70,000 Da Particle MMAD (GSD): 4.0 µm (1.5) Respirable fraction: 35%	SPF F344 rat, female (24/group)	Whole body inhalation (nose only for tracer exposure) 0, 10, 40 mg/m^3 aerosol 3 mo. (6 hr/d, 5 d/wk) 15 mo. recovery	10 mg/m^3 ↑ total protein in BALF ↓ tracer clearance rate (68-93% of control) 40 mg/m^3 ↑ PMNs, macrophages, LDH, β-glucuronidase, and total protein in BALF ↓ tracer clearance rate (20-63% of control)	Bellman 1992
ACUDYNE™ Shine Polymer (39-41% styrene/acrylates copolymer) and ACUDYNE ™ Bold Polymer MW: not reported Particle MMAD (GSD): not reported Respirable fraction: not reported	Rat (strain, sex, and group size not reported)	Nose only inhalation 10.8, 100 mg/mg^3 2 wk (frequency not reported	10.8 mg/m^3 None reported 100 mg/m^3 Slight irritant effects	Dow Chemical (undated) as cited in CIR 2014
ACUDYNE™ Shine Polymer (39-41% styrene/acrylates copolymer) and ACUDYNE ™ Bold	Rat (strain, sex, and group size not reported)	Nose only inhalation Concentrations not reported	NOAEL reported to be 8.3 mg/m^3 based on changes in lung and lymph nodes.	Dow Chemical (undated) as cited in CIR 2014

Table [SEQ Table * ARABIC]. Inhalation studies of lung overload from HMW polymers in laboratory animals.

Test Material[a]	Animals	Exposure and Recovery	Lung effects	Reference
Polymer MW: not reported Particle MMAD (GSD): not reported Respirable fraction: not reported		13 wk (frequency not reported		
butyl acrylate/ methacrylic acid polymer MW: not reported Particle MMAD (GSD): not reported Respirable fraction: not reported	Rat (strain, sex, and group size not reported)	Inhalation (method not reported) 0, 1, 10, 30 mg/m^3 aerosol 3 mo. (frequency not reported) 6 wk recovery	1 mg/m^3 None reported 10 mg/m^3 None reported 30 mg/m^3 ↑ ("high") Incidence alveolar histiocytosis	Evans et al., 1998 [as cited in Norris and Tyler, 2000]
9000 Toner MW: 70,000 Da Particle MMAD (GSD): 4.0-4.1 µm (1.3) Respirable fraction: 34%	Syrian Golden Han:AURA hamster, male and female, (41 M and 24 F/group for main study)	Whole body inhalation 0, 4, 16, 64 mg/m^3 aerosol 90 d (6 hr/d, 5 d/wk) Up to 100 d recovery	4 mg/m^3 ↑ absolute and/or relative lung weight ↑ incidence accumulation of particle-laden alveolar macrophages 16 mg/m^3 ↑ absolute and/or relative lung weight ↑ incidence lung/LALN histopathology ("slight appearance of particles" in LALN; accumulation of particle-laden alveolar macrophages; very slight septal thickening due to hypercellularity and interstitial inflammatory cell infiltrate) ↑ grey/black areas of lungs (retained test material) 64 mg/m^3 ↑ absolute and/or relative lung weight ↑ incidence lung/LALN histopathology ("slight appearance of	Fraunhofer Institute, 1988 (Unpublished report) There were 19 unscheduled sacrifices unrelated to treatment; in addition, 30% of the animals (mostly males) exhibited wet tail disease

Table [SEQ Table * ARABIC]. Inhalation studies of lung overload from HMW polymers in laboratory animals.

Test Material[a]	Animals	Exposure and Recovery	Lung effects	Reference
			particles" in LALN; accumulation of particle-laden alveolar macrophages; very slight septal thickening due to hypercellularity and interstitial inflammatory cell infiltrate) ↑ grey/black areas of lungs (retained test material), discolored LALN	
Toner A (styrene/butylmethacrylate random copolymer) MW: Not reported Particle MMAD (GSD): 4.0 µm (1.5) Respirable fraction: 35%	F344/CrlBR rat, female, (58-66/group)	Whole body inhalation 0, 4, 16, 64 mg/m^3 aerosol 3 mo. (6 hr/d, 5 d/wk) Up to 6 mo. recovery	4 mg/m^3 ↑ incidence slight to moderate accumulation particle-laden macrophages 16 mg/m^3 ↑ incidence very slight interstitial fibrosis in lungs ↑ incidence slight to moderate accumulation particle-laden macrophages in lungs ↑ incidence slight interstitial inflammatory cell infiltration in lungs ↓ tracer clearance rate (half-time 1.3X to 1.7X control) 64 mg/m^3 ↑ LDH, β-glucuronidase, total protein, hydroxyproline, and PMN count in BALF ↑ absolute wet and dry lung weights, lymph node weights ↑ very slight interstitial fibrosis in lungs ↑ slight to moderate accumulation particle-laden macrophages in lungs ↑ slight interstitial inflammatory cell infiltration ↑ alveolar PMN infiltration ↑ focal/multifocal alveolar type-II cell hyperplasia ↓ tracer clearance rate (half-time 2.1X to 8.8X control) ↑ grey/black areas of lungs (retained test material), discolored LALN	Fraunhofer Institute, 1991a. (Unpublished report) There were 17 unscheduled sacrifices unrelated to treatment.
Toner B (styrene/ butadiene random copolymer) MW: not reported Particle MMAD (GSD): 4.0-	F344/CrlBR rat, female (50/group for main study, 10/group for clearance)	Whole body inhalation 0, 1, 4, 16, or 64 mg/m^3 aerosol	1 mg/m^3 None reported 4 mg/m^3 None reported 16 mg/m^3	Fraunhofer Institute, 1991b. (Unpublished report) There were 3 unscheduled sacrifices unrelated

Table [SEQ Table * ARABIC]. Inhalation studies of lung overload from HMW polymers in laboratory animals.

Test Material[a]	Animals	Exposure and Recovery	Lung effects	Reference
4.2 µm (1.5-1.8) Respirable fraction: 35.4-37.2%		3 mo. (6 hr/d, 5 d/wk) Up to 6 mo. recovery	↑ grey/black areas of lungs (retained test material) 64 mg/m^3 ↑ LDH, β-glucuronidase, and PMNs in BALF ↑ enlargement of LALN ↑ very slight to slight focal/multifocal alveolar type II cell hyperplasia ↑ very slight to slight focal/multifocal interstitial inflammatory cell infiltration in lungs ↑ very slight to slight interstitial fibrosis in lungs ↑ slight to moderate particle deposition in LALN ↑ slight to moderate lymphoid hyperplasia in LALN ↓ tracer clearance rate (half-time 1.2X to 2.2X control) ↑ grey/black areas of lungs (retained test material), discolored LALN	to treatment.
9000 Toner (styrene/butylmethacrylate random copolymer) MW: 70,000 Da Particle MMAD (GSD): 4.0 µm (1.6-1.8) Respirable fraction: 36.5-37.7%	Syrian Golden Han:AURA hamster, male and female, (50/group)	Whole body inhalation 0, 1.5, 6, or 24 mg/m^3 (mo. 1-5); 0, 4, 16, or 64 mg/m^3 (mo. 6-18) aerosol 18 mo. (6 hr/d, 5 d/wk) 3-5 mo. recovery	1.5/4.0 mg/m^3 ↑ bronchiolar/alveolar hyperplasia in males ↑ accumulation particle-laden macrophages ↑ interstitial inflammatory cell infiltration in males ↑ lymphatic hyperplasia in LALN in males ↑ particle deposits in LALN 6/16.0 mg/m^3 ↑ interstitial fibrosis ↑ bronchiolar/alveolar hyperplasia ↑ accumulation particle-laden macrophages ↑ alveolar PMN infiltration ↑ interstitial inflammatory cell infiltration ↑ lymphatic hyperplasia in LALN ↑ particle deposits in LALN 24/64 mg/m3 ↓ % lymphocytes; ↑% and absolute count neutrophils in blood ↑ absolute and relative lung weight ↑ total cell number, PMNs, macrophages, LDH, β-glucuronidase, total protein, and hydroxyproline in BALF ↑ interstitial fibrosis	Fraunhofer Institute, 1991c (Unpublished report)

Table [SEQ Table * ARABIC]. Inhalation studies of lung overload from HMW polymers in laboratory animals.

Test Material[a]	Animals	Exposure and Recovery	Lung effects	Reference
			↑ bronchiolar/alveolar hyperplasia ↑ accumulation particle-laden macrophages ↑ alveolar PMN infiltration ↑ interstitial inflammatory cell infiltration ↑ lymphatic hyperplasia in LALN ↑ particle deposits in LALN ↓ tracer clearance rate (half-time 1.7 to 3.1X control) in males	
Ultrafine Kevlar Aramid synthetic fibrils MW: not reported Particle MMAD (GSD): <2 µm (GSD not reported) Respirable fraction: >70%	Crl:CD(SD)BR rat, male and female (100/sex/group)	Whole body inhalation 0, 0.08, 0.32, 0.63, or 2.23 mg/m^3 (0, 2.5, 25, 100, or 400 fibers/cc) 24 mo. (6 hr/d, 5 d/wk) 12 mo. recovery (2.23 mg/m^3 group only)	0.08 mg/m^3 ↑ slight dust cell (macrophage) response 0.32 mg/m^3 ↑ dust cell (macrophage) response ↑ foamy macrophage response ↑ hyperplasia of type II pneumocytes ↑ collagenized fibrosis ↑ alveolar bronchiolization 0.63 mg/m^3 ↑ lung weight ↑ dust cell (macrophage) response ↑ foamy macrophage response ↑ hyperplasia of type II pneumocytes ↑ collagenized fibrosis ↑ alveolar bronchiolization ↑ cholesterol granuloma in females 2.23 mg/m^3 ↑ mortality due to obliterative bronchiolitis (29 M and 14 F) ↑ lung weight ↑ dust cell (macrophage) response ↑ foamy macrophage response ↑ hyperplasia of type II pneumocyte ↑ collagenized fibrosis ↑ alveolar bronchiolization ↑ cholesterol granuloma in females ↑ centriacinar emphysema ↑ keratinized cystic squamous cell carcinoma in females (6/56)	Lee et al. 1988

Table [SEQ Table * ARABIC]. Inhalation studies of lung overload from HMW polymers in laboratory animals.

Test Material[a]	Animals	Exposure and Recovery	Lung effects	Reference
Polyvinyl chloride (PVC) powder MW: Not reported Particle MMAD (GSD): 1.3 µm (2.07) Respirable fraction: not reported	Rat, female (strain not reported); group sizes not reported	Inhalation (method not reported) 0, 3.3, 8.3 or 20.2 mg/m^3 7 mo. (25 hr/wk) 15-100 d recovery	3.3 mg/m^3 ↓ tracer clearance rate (mean half-time 1.2X control) 8.3 mg/m^3 ↓ tracer clearance rate (mean half-time 2.1X control) 20.2 mg/m^3 ↓ tracer clearance rate (mean half-time 3.2X control)	Muhle et al., 1990b (21: 374)
9000 Toner (styrene/butylmethacrylate random copolymer) MW: 70,000 Da Particle MMAD (GSD): 4.0 µm (1.5) Respirable fraction: 35%	SPF F344 rat, male and female (56-74/sex/group)	Whole body inhalation (nose only for tracer exposure) 0, 1, 4, 16 or 64 mg/m^3 aerosol 3 mo. (6 hr/d, 5 d/wk) 3 mo. recovery	1 mg/m^3 None reported 4 mg/m^3 None reported 16 mg/m^3 ↑ tachypnea ↑ relative lung weight in males ↑ slight LALN enlargement ↑ lung histopathology (particle-laden macrophages, few particles found in alveolar walls; slight degree of thickening of the alveolar structure due to hypertrophy and hyperplasia of Type II cells and accumulation of a few interstitial cells) 64 mg/m^3 ↑ tachypnea ↑ absolute and relative lung weights ↑ slight LALN enlargement ↑ lung histopathology (particle-laden macrophages, few particles found in alveolar walls; slight degree of thickening of the alveolar structure due to hypertrophy and hyperplasia of Type II cells and accumulation of a few interstitial cells)	Muhle et al., 1990a (2: 341)
9000 Toner (styrene/butylmethacrylate	SPF F344 rats, male and female	Whole body inhalation	1 mg/m^3 ↑ occasional particle-laden macrophages	Muhle et al., 1991 (17: 280); Bellmann

Table [SEQ Table * ARABIC]. Inhalation studies of lung overload from HMW polymers in laboratory animals.

Test Material[a]	Animals	Exposure and Recovery	Lung effects	Reference
random copolymer) MW: 70,000 Da Particle MMAD (GSD): 4.0 µm (1.5) Respirable fraction: 35%	(100/sex/group in main study)	(nose only for tracer exposure) 0, 1, 4, or 16 mg/m^3 aerosol 24 mo. (6 hr/d, 5 d/wk) Up to 2 mo. recovery	4 mg/m^3 ↑ PMN and lymphocytes in BALF ↑ particle-laden macrophages ↑ foamy macrophage accumulation, very slight ↑ pulmonary fibrosis, minimal to mild ↓ tracer clearance rate (mean half-time 1.2X – 2.3X control) 16 mg/m^3 ↓ lung volume and compliance ↑ absolute and relative lung weights ↓ macrophages, ↑ PMN, lymphocytes, LDH, β-glucuronidase and protein in BALF ↑ particle-laden macrophages ↑ foamy macrophage accumulation, slight ↑ bronchioalveolar hyperplasia, slight to moderate ↑ alveolar squamous cell metaplasia in some females ↑ cholesterol granulomas (occasional) ↑ alveolar lipoproteinosis, very slight to slight ↑ pulmonary fibrosis, mild to moderate ↑ lymphoid hyperplasia of LALN ↓ tracer clearance rate (mean half-time 2.1X -6.6X control)	et al., 1991; Muhle et al., 1989; Heinrich et al., 1989
ADR (acrylic latex consisting of ethyl acrylate, methacrylic acid, methyl methacrylate, acrylic acid polymer) MW: not reported Particle MMAD (GSD): 2.64-3.09 µm (3.54-3.90) Respirable fraction: not reported	Sprague-Dawley rat, male and female (10-15/sex/group)	Whole body inhalation 0, 30, 100 or 300 mg/m^3 aerosol 3 mo. (2 hr/d, 5 d/wk) 6 wk recovery (5 females only)	30 mg/m^3 None reported 100 mg/m^3 ↑ alveolar histiocytosis ↑ lymphadenitis in mediastinal lymph nodes ↑ interstitial pneumonitis in recovery females 300 mg/m^3 ↑ absolute and relative lung weights ↑ alveolar histiocytosis ↑ lymphadenitis in mediastinal lymph nodes ↑ intra-alveolar cellular debris in recovery females ↑ interstitial pneumonitis in recovery females	Norris and Tyler, 2000

Table [SEQ Table * ARABIC]. Inhalation studies of lung overload from HMW polymers in laboratory animals.

Test Material[a]	Animals	Exposure and Recovery	Lung effects	Reference
HMDI-based polyurethane/ polyurea polymer MW: >20,000 Da Particle MMAD (GSD): 1.00-1.54 µm (1.86-1.94) Respirable fraction: 4.3-6.1% (modeled)	SPF Wistar rat, male and female (10-15F and 22M/group)	Nose only inhalation 0, 5, 26 or 107 mg/m^3 13 wk (6 hr/d, 5 d/wk) 4 wk recovery	5 mg/m^3 None reported 26 mg/m^3 ↑ absolute wet lung weight ↑ total cell count, MCV, alveolar macrophage and PMN counts, total protein, LDH and GGT in BALF ↑ incidence mucus and/or cells in the trachea and pulmonary airways; enlarged and/or foamy macrophages in lung; hypercellularity of the bronchiolo-alveolar junction; polymer/debris-laden macrophages in LALNs 107 mg/m3 ↑ absolute wet lung weight ↑ total cell count, MCV, alveolar macrophage and PMN counts, neutrophil count, total protein, LDH and GGT in BALF ↑ incidence mucus and/or cells in the trachea and pulmonary airways; enlarged and/or foamy macrophages in lung; hypercellularity of the bronchiolo-alveolar junction; polymer/debris-laden macrophages in LALNs	Pauluhn, 2014
HMDI-based polyurethane/ polyurea polymer MW: >20,000 Da Particle MMAD (GSD): 0.9-1.4 µm (2.2-2.5) Respirable fraction: not reported	SPF Wistar rat, male (6-12/group)	Nose only inhalation 0, 5, 22, or 121 mg/m^3 2 wk (6 hr/d, 5 d/wk) 2 wk recovery	5 mg/m^3 None reported 22 mg/m^3 ↑ MCV in BALF Minimal changes in alveolar macrophage morphology 121 mg/m^3 ↑ total cell count, MCV, alveolar macrophage and PMN counts, total protein, LDH, and GGT in BALF ↑ absolute lung wet weight ↑ incidence hyperplastic and hypertrophic alveolar macrophages (alveolar histiocytosis) containing foamy-brownish cytoplasmatic inclusions ↑ incidence hypercellularity/epithelial thickening at the bronchiolo-alveolar junction with inflammatory infiltrates, increased numbers of alveolar macrophages, and focal septal thickening	Pauluhn, 2014

Table [SEQ Table * ARABIC]. Inhalation studies of lung overload from HMW polymers in laboratory animals.

Test Material[a]	Animals	Exposure and Recovery	Lung effects	Reference
HMDI-based polyurethane/ polyurea polymer MW: >20,000 Da Particle MMAD (GSD): not reported Respirable fraction: not reported	SPF Wistar rat, male (18/group)	Nose only inhalation 0, 57, or 979 mg/m^3 6 hr 1 wk recovery	57 mg/m^3 ↑ PMN count and GGT in BALF 979 mg/m^3 ↓ body weight ↑ frequency of transient respiratory effects and hypothermia ↑ absolute lung wet weight ↑ total cell count, MCV, alveolar macrophage count, PMN count, total protein, LDH, and GGT in BALF	Pauluhn, 2014
Acrylates copolymer (n-butyl acrylate, methyl methacrylate, and methacrylic acid) MW not reported Particle MMAD (GSD): 2.4-2.5 µm (GSD not reported) Respirable fraction: not reported	Crl:CD(SD)BR rat, male and female (15/sex/group)	Whole body inhalation 0, 1, 10, 30 mg/m^3 (as formulation) or 0.185, 1.67, or 4.94 mg/m^3 (polymer) 13 wk (6 hr/d, 7 d/wk) 4 wk recovery	1/0.85 mg/m^3 None reported 10/1.67 mg/m^3 None reported 30/4.94 mg/m^3 ↑ lung weight ↑ alveolar histiocytosis with focal macrophage accumulation in alveolar spaces	WIL Research Laboratories, Inc., 1997 (as cited in CIR, 2002)

↑ = statistically or biologically significant increase; ↓= statistically or biologically significant decrease; LALN = lung-associated lymph nodes; BALF = broncho-alveolar lavage fluid; LDH – lactate dehydrogenase; GGT = γ glutamyltransferase; MCV = mean cellular volume of lavageable cells; PMN = polymorphonuclear neutrophils

[a] TEST MATERIAL DETAILS:
9000 Toner: 9000-type xerographic toner material composed of about 90% 58:42 styrene/butylmethacrylate random copolymer (CAS no. 25213-39-2) and 10% high-purity furnace-type carbon black (CAS no. 7440-44-0)

Toner A; 1075 Toner Fines; composition corresponds to 1075 xerographic toner. >90% random copolymer (CASRN 25213-39-2); 5-10% high purity furnace black (CASRN 1333-86-4); ~2% quaternary ammonium salt cetyl pyridinium chloride (CASRN 123-03-5)

Toner B; S090 Toner Fines; composition corresponds to 5090 xerographic toner. 75-85% random copolymer (styrene/butadiene; CASRN 9003-55-8); <5%

Table [SEQ Table * ARABIC]. Inhalation studies of lung overload from HMW polymers in laboratory animals.

Test Material[a]	Animals	Exposure and Recovery	Lung effects	Reference

high purity furnace black (CASRN 1333-86-4); 15-20% iron oxide (CASRN 1309-37-1); <2% quaternary ammonium salts (CASRNs 3843-16-1 and 123312-54-9)

Aqueous dispersion resin (ADR) is a water-based acrylate copolymer supplied by Amerchol Corporation (lot 10-19-92; Edison, NJ) containing 26% of an acrylic latex consisting of ethyl acrylate, methacrylic acid, methyl methacrylate, acrylic acid polymer (CAS RN 25053-63-8), formulated in 73% water neutralized to pH 7 with 1% salts and surfactants

HDMI-based polyurethane-polyurea polymer. Poorly soluble, slowly biodegradable linear anionic hexamethylene diisocyanate monomer-based polyurethane-polyurea HMW polymer of >20,000 Da incorporating both hydrophilic and hydrophobic segments. When dispersed in water, the insoluble content of dispersion was approximately 30%.

Acrylates copolymer (n-butyl acrylate, methyl methacrylate, and methacrylic acid). Vehicle and polymer formulation had 69% ethanol (16.2% solids by wt, viscosity 16 cPs, and pH 8.4). Contained monomer levels of 5 ppm n-butyl acrylate, 33 pm methyl methacrylate, and 15.7 methacrylic acid.

3. BENCHMARK DOSE (BMD) MODELING OUTPUTS

EPA's BMD software (BMDS, 3.1.1) was used for dose-response modeling of dichotomous data. All dichotomous models in the software were considered. A benchmark response (BMR) of 10% extra risk was employed, and model fit was evaluated using the χ^2 goodness-of-fit p-value ($p > 0.1$), magnitude of scaled residuals at doses near the BMR, and visual assessment of the model fit as displayed graphically. The BMCL from the model with the lowest Akaike's Information Criterion (AIC) was chosen from among all models providing adequate fit.

Muhle et al. 1991; Bellmann et al., 1991; Muhle et al., 1989; Heinrich et al., 1989

The incidence of pulmonary fibrosis (all severity levels, minimal to moderate) in rats exposed to 9000 type print toner for 2 years (6 hours/day, 5 days/week) was subjected to BMD modeling. The modeled data are shown in [REF _Ref46549694 \h * MERGEFORMAT] below.

Table [SEQ Table * ARABIC]. Incidence of pulmonary fibrosis in rats exposed to 9000 print toner (21-26-month sacrifices).

Exposure concentration (mg/m^3)	Number exposed[a]	% Affected	Number affected
0	90	1.2	1
1	90	0	0
4	90	21.6	19
16	90	92.1	83

[a] Number per group was reported as "about 90 animals/exposure group". Number affected was calculated as % affected (summed across severity groups) x 90 and rounded to nearest integer.

Source: Muhle et al. 1991

Models providing adequate fit (χ^2 $p > 0.1$) to the fibrosis incidence data included gamma, log-logistic, multistage (2- and 3-degree), and log-probit. Among these, the log-probit model had the lowest AIC and was selected. The BMC and BMCL predicted by the log-probit model were 3.0 and 2.5 mg/m^3, respectively. BMD model output and graphical display of the data fit for the log-probit model are shown below.

A. BMD Model Output for Selected Model (Log-Probit) for Pulmonary Fibrosis

Frequentist Log-Probit Unrestricted Option Set #1

User Input

Info	
Model	frequentist Log-Probit v1.1
Dataset Name	Muhle1991_toner_fibrosis_2yr
User notes	[Add user notes here]

Model Options	
Risk Type	Extra Risk
BMR	0.1
Confidence Level	0.95
Background	Estimated

Model Data	
Dependent Variable	[Dose]
Independent Variable	[Incidence]
Total # of Observations	4

Model Results

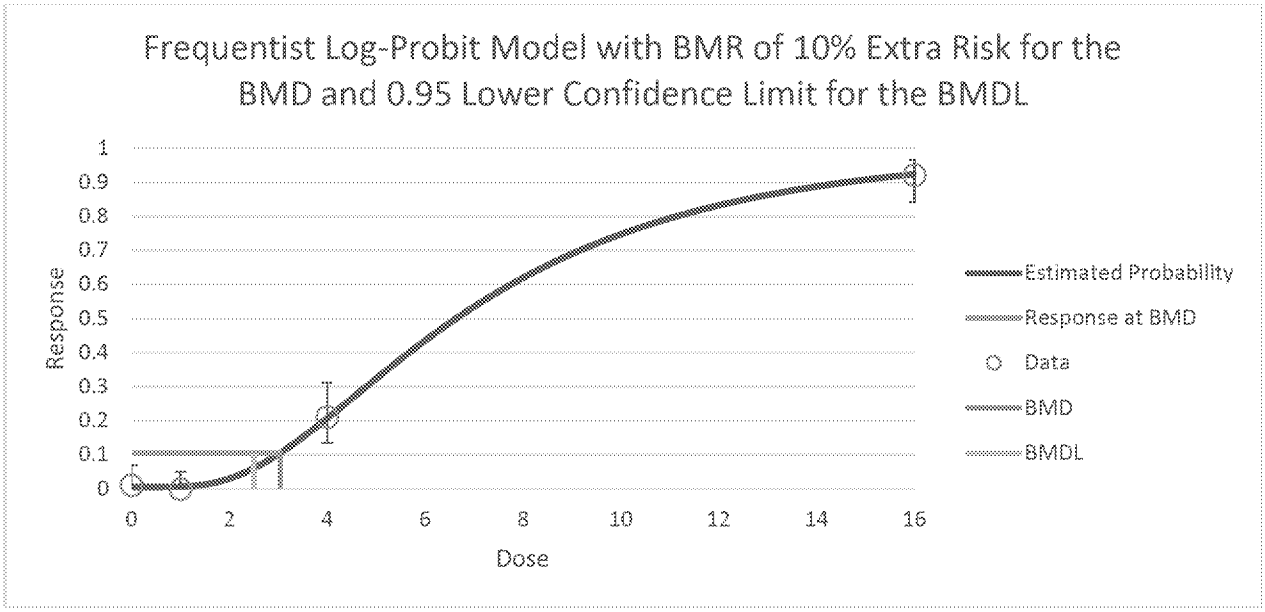
Benchmark Dose	
BMD	3.03602163
BMDL	2.495470955
BMDU	3.583395138
AIC	160.5429675
P-value	0.294321824
D.O.F.	1
Chi^2	1.099746133

Model Parameters	
# of Parameters	3
Variable	Estimate
g	0.005598734
a	-3.09242313
b	1.630610825

Goodness of Fit					
Dose	Estimated Probability	Expected	Observed	Size	Scaled Residual
0	0.005598734	0.503886102	1	90	0.700865
1	0.006585825	0.59272426	0	90	-0.772434
4	0.207191742	18.64725679	19	90	0.0917418
16	0.923867598	83.14808379	83	90	-0.058857

Analysis of Deviance					
Model	Log Likelihood	# of Parameters	Deviance	Test d.f.	P Value

Full Model	-76.48022106	4	-	-	-
Fitted Model	-77.27148375	3	1.58252536	1	0.2083973
Reduced Model	-215.5078117	1	278.055181	3	<0.0001



Lee et al. 1988

Several lung lesions were significantly increased by 24-month exposure to Kevlar fibrils at the LOAEC mass/volume concentration of 0.32 mg/m^3 (Lee et al., 1988). Incidences of all lesions affected at the LOAEC are shown in [REF _Ref46550170 \h * MERGEFORMAT] below. Among the five lesion types increased at the LOAEC, two were selected for BMD modeling (shown in bold in [REF _Ref46550170 \h * MERGEFORMAT]): foamy macrophage response and alveolar bronchiolarization. The remaining three lesions exhibited "all or none" dose-response relationships that are not amenable to BMD modeling.

Table [SEQ Table * ARABIC]. Incidence of pulmonary pathology[a] in rats exposed to Kevlar fibrils for 24 months.

Exposure concentration (mg/m^3)	Number exposed	Dust cell (macrophage) response	Foamy macrophage response	Type II pneumocyte hyperplasia	Fibrosis, collagenized	Alveolar bronch-iolarization
Male						
0	69	0	**7**	0	0	**0**
0.08	69	1	**2**	1	0	**0**
0.32	67	65	**21**	65	67	**37**
0.63	68	67	**47**	67	67	**48**
2.32	36	32	**18**	32	35	**16**
Female						
0	68	0	**4**	0	0	**0**
0.08	64	0	**3**	0	0	**1**
0.32	65	63	**20**	63	57	**51**
0.63	69	68	**65**	68	65	**68**
2.32	56	54	**51**	54	54	**52**

[a] Bolded column indicate data subjected to BMD modeling

Source: Lee et al., 1988

[REF _Ref46550526 \h * MERGEFORMAT] presents the BMD modeling results for pulmonary pathology in rats exposed to Kevlar fibrils. As the Table shows, no model fit was achieved when all dose groups were included in modeling of incidences (either lesion) in males. With the high dose group omitted, the 2-degree multistage model was the only model providing adequate fit to the incidences of foamy macrophages in males; this model provided BMC and BMCL estimates of 0.19 and 0.15 mg/m^3, respectively. For alveolar bronchiolarization in males, no model fit was achieved with the high dose group omitted; with the two highest dose groups omitted, all but the 1-degree multistage model provided adequate fit, and the dichotomous Hill model had the lowest AIC. The BMC and BMCL predicted by this model were 0.28 and 0.09 mg/m^3, respectively.

For incidences of both foamy macrophages and alveolar bronchiolarization in female rats, the dichotomous Hill model was the only model providing adequate fit to the full datasets. This model yielded BMC and BMCL estimates of 0.28 and 0.24 mg/m^3 for foamy macrophages and 0.13 and 0.10 mg/m^3 for alveolar bronchiolarization in female rats.

Table [SEQ Table * ARABIC]. BMD Modeling Results for Pulmonary Pathology in Rats Exposed to Kevlar Fibrils for 2 years.

Sex - Lung Lesion Type	Dataset	Selected model	BMC (mg/m^3)	BMCL (mg/m^3)
Male – Foamy macrophages	All	No model fit		
	HDD	Multistage 2 degree	0.19	0.15
Male – Alveolar bronchiolarization	All	No model fit		
	HDD	No model fit		
	2HDD	Dichotomous Hill	0.28	0.09
Female – Foamy macrophages	All	Dichotomous Hill	0.28	0.24
Female – Alveolar bronchiolarization	All	Dichotomous Hill	0.13	0.10
BMC = benchmark concentration; BMCL = lower confidence limit on benchmark concentration; HDD = highest dose group omitted; 2HDD = 2 highest dose groups omitted.				

The lowest BMCL shown in the table is 0.09 mg/m^3; however, because this BMCL was obtained only by dropping two of the four exposure groups, and was very close to the BMCL of 0.1 mg/m^3 obtained by modeling alveolar bronchiolarization in females using all exposure groups, the latter was selected as the best POD for this study. BMD model output and graphical display of the female alveolar bronchiolarization data fit for the dichotomous Hill model are shown below.

B. BMD Model Output for Selected Model (Dichotomous Hill) for Female Alveolar Bronchiolarization

Frequentist Dichotomous Hill Unrestricted Option Set #1

User Input

Info	
Model	frequentist Dichotomous Hill v1.1
Dataset Name	Lee1988_synfibers_bronchiol_2yr_females
User notes	[Add user notes here]

Model Options	
Risk Type	Extra Risk
BMR	0.1
Confidence Level	0.95
Background	Estimated

Model Data	
Dependent Variable	[Dose]
Independent Variable	[Incidence]
Total # of Observations	5

Model Results

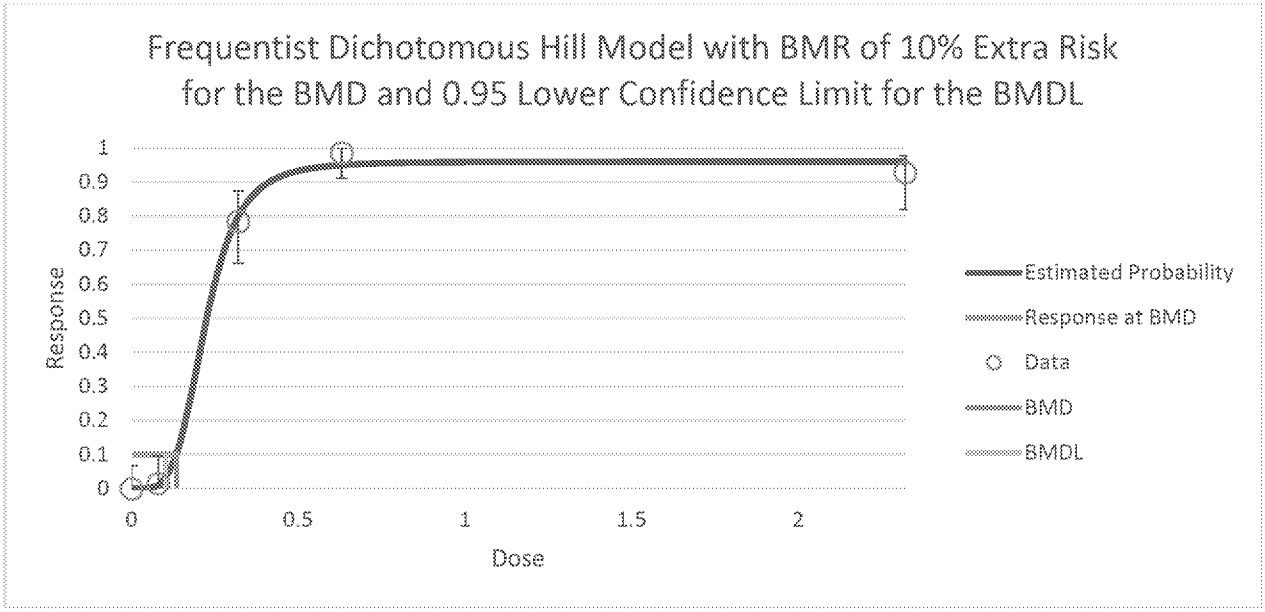
Benchmark Dose	
BMD	0.134448091
BMDL	0.102215165
BMDU	0.174500488
AIC	127.1745156
P-value	0.176399296
D.O.F.	2

Chi^2	3.470010254

Model Parameters	
# of Parameters	4
Variable	Estimate
g	Bounded
v	0.960289295
a	6.528285835
b	4.325966045

Goodness of Fit					
Dose	Estimated Probability	Expected	Observed	Size	Scaled Residual
0	1.523E-08	1.03564E-06	0	68	-0.001018
0.08	0.011670656	0.746921978	1	64	0.2945547
0.32	0.798861796	51.92601671	51	65	-0.286536
0.63	0.950042444	65.55292861	68	69	1.3522282
2.32	0.960252474	53.77413855	52	56	-1.213517

Analysis of Deviance					
Model	Log Likelihood	# of Parameters	Deviance	Test d.f.	P Value
Full Model	-58.65296117	5	-	-	-
Fitted Model	-60.58725782	3	3.8685933	2	0.1445259
Reduced Model	-222.4412535	1	327.576585	4	<0.0001



4. MPPD Modeling Outputs

The predictions for Muhle *et al.* (1990) shown in [REF _Ref46769100 \h], below, are specific to those experimental parameters only, so we conducted additional simulations to impart appreciation of why they should be conducted with exact particle exposure characteristics, experimental conditions (concentration, duration), and species parameters for any polymer undergoing evaluation.

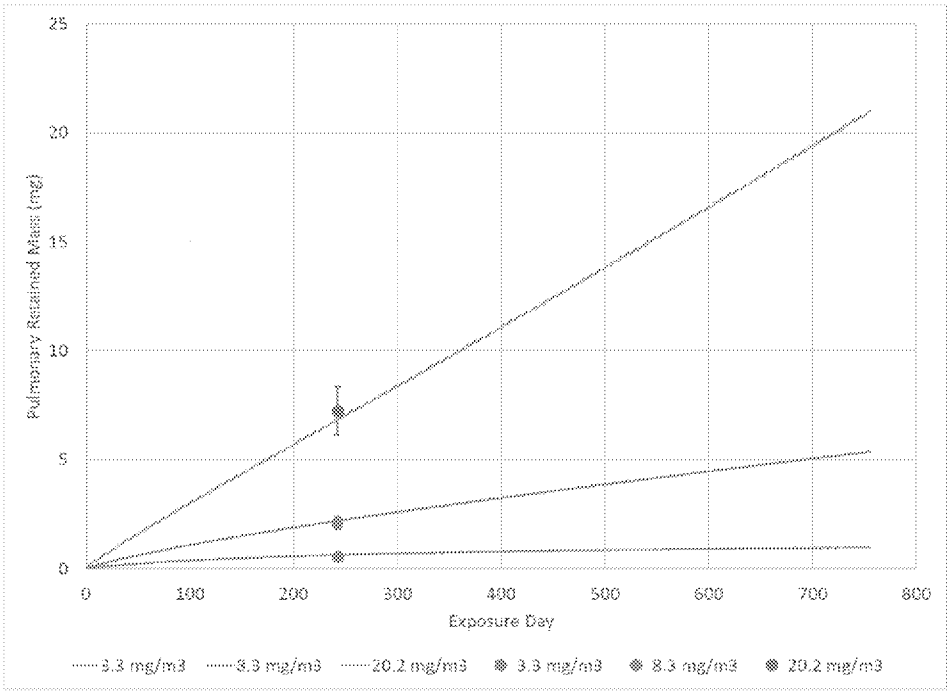


Figure [SEQ Figure * ARABIC]. MPPD predictions for retained PU mass in F344 rats under the exposure conditions for the Muhle *et al.* (1990) study**.** Simulations were performed to characterize the 8-month study with a particle MMAD size of 1.3 µm, a GSD of 2.07, and a density of 1.3 g/cm^3 for three concentrations (3.3, 8.3, and 20.2 mg/m^3). Experimental data for PU burdens are shown as solid circles with standard deviation and the predictions as solid lines for different concentrations.

Additional simulations were conducted at the same three exposure concentrations as Muhle *et al.* (1990), but the following three key input parameters were varied and bounded based on the rationale provide below.

1) MMAD: 0.1, 2.5, and 10 µm. This represents a range of particle sizes covering different dominant deposition mechanisms across species and covers size range of particles for which overload was demonstrated in Muhle *et al.* (1990). [REF _Ref46769370 \h * MERGEFORMAT] illustrates the impact of predicted retained PU mass (mg) for simulations for particles sizes of MMAD at 0.1, 2.5, and 10 µm.

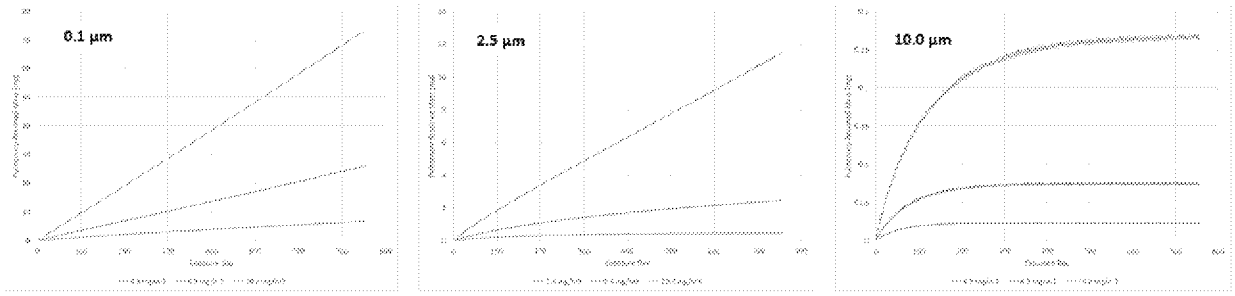


Figure [SEQ Figure * ARABIC]. Impact of particle size on predicted inhaled retained mass burden in PU region of F344 rats. Simulations were performed under the same exposure conditions as for the Muhle *et al.* (1990) study with an exposure of 5 h /d and 5 d/w with a particle GSD of 2.07 and density of 1.3 g/cm^3; and at the same concentrations. The three panels show predicted retained PU mass (mg) for an MMAD of 0.1 µm (left), 2.5 µm (middle), and 10.0 µm (right); note the y-axis is different for each.

At the largest particle size, overload is not predicted to be achieved in the rat. For the particle size with an MMAD of 2.5 µm, slightly larger than the MMAD of 1.3 µm in the Muhle *et al.* (1990) study, overload is only evident at the highest exposure concentration; whereas for the MMAD of 0.1 µm, predictions indicate overload would occur in the rats at all three exposure concentrations. There are also associated substantial differences in the predicted mass with a 400% increase in PU burden at the lowest particle size; whereas at the larger particles sizes with an MMAD of 2.5 and 10 µm, predictions for retained PU mass are decreased by 50-60% and two orders of magnitude (99%), respectively.

2) GSD: 1, 2.07, and 3 with an MMAD of 3.5 µm. If an exposure aerosol has a GSD greater than 3, it calls into question the quality of the control on exposure generation and characterization. This range includes the GSD of 2.07 used in the Muhle *et al.* (1990) study. We chose a particle size of 3.5 µm with the same density of the PVC particles in the Muhle *et al.* (1990) study to illustrate how filtering due to impaction mechanisms for this particle size in the extrathoracic (ET) region of the respiratory tract influences what penetrates to the lower respiratory tract for deposition and subsequent clearance. A large GSD near that size range can substantively impact retained PU mass results. [REF _Ref46769955 \h * MERGEFORMAT] illustrates the impact of variation in the GSD for an aerosol with particle size MMAD of 3.5 µm. At the larger GSD, there is a 15% predicted decrease in the retained PU mass but when the aerosol is monodisperse with a GSD of 1.0, there is a 50% increase in the predicted retained PU mass and overload most evident at the highest concentration. The decrease in predicted retained PU mass at the larger GSD illustrates the filtering effect in the ET region. We also ran simulations with an MMAD of 1.3 µm as in the Muhle *et al.* (1990) study for a GSD of 1.0 and 3.0.

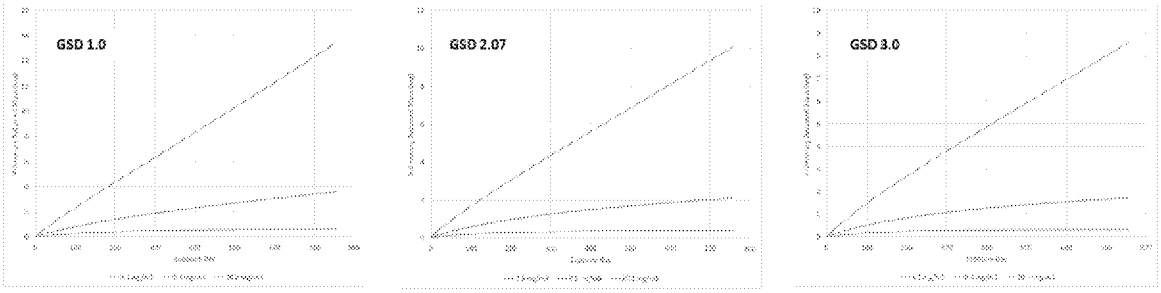


Figure [SEQ Figure * ARABIC]. Impact of geometric standard deviation (GSD) on predicted inhaled retained mass burden in PU region of F344 rats for a particle exposure with an MMAD of 3.5 μm. Simulations were performed under the same exposure conditions (5 h/d and 5 d/w) as for the Muhle *et al.* (1990) study, including a density of 1.3 g/cm^3 and at the same concentrations. The three panels show predicted retained PU mass (mg) for a GSD of 1.0 (left), 2.08 (middle), and 3.0 (right); note the y-axis is different for each. The middle GSD is the same as that in the Muhle *et al.* (1990) study.

For this particle size, a 5% decrease is instead predicted at a GSD of 1.0 and an increase of 5% at the larger GSD of 3.0. These simulations illustrate and reinforce the need for exposure specific data for determining these input parameters.

3) Density: 0.85 and 2.0 g/cm^3. This span of density between 0.85 and 2.0 g/cm^3 covers the range of density based on values for plastic polymers provided in Lambert and Wagner (2017). Simulations used all other input parameters the same as in the Muhle *et al.* (1990) study. [REF _Ref46769919 \h * MERGEFORMAT] shows predictions for each of these density values at the three concentrations. The predicted retained PU burdens in rats are 5-10% less than at the density of 1.3 g/cm^3 shown in Figure 2, whereas a 10% increase in retained PU burden is predicted for the higher density. Predicted overload is approximately the same for each.

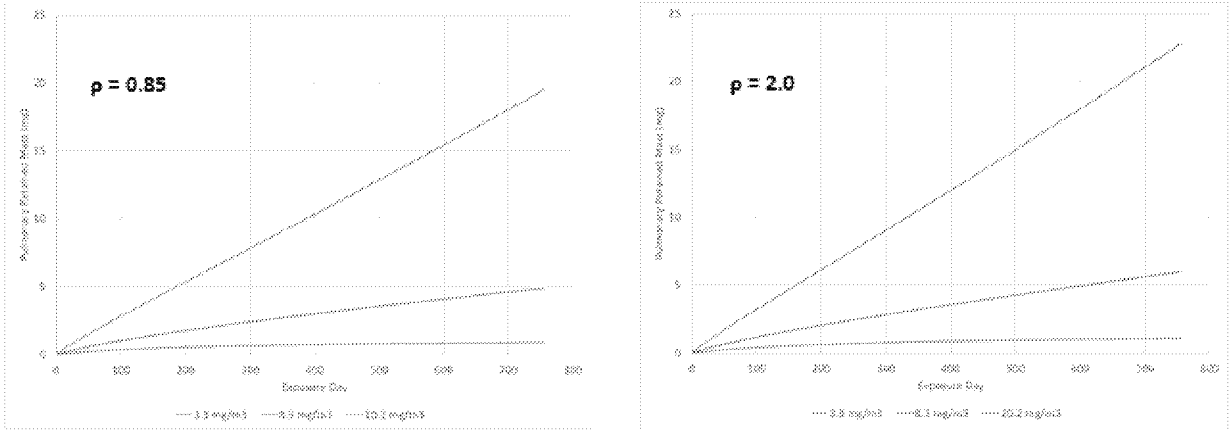


Figure [SEQ Figure * ARABIC]. Impact of particle density (ρ) on predicted inhaled retained mass burden in PU region of F344 rats. Simulations were performed under the same exposure conditions as for the Muhle *et al.* (1990) study with an exposure of 5 h /d and 5 d/w with a particle MMAD of 1.3 μm and GSD of 2.07; and at the same concentrations. The panel on the

left is for $\rho = 0.85$ g/cm^3 and the right for $\rho = 2.0$ g/cm^3, values which bracket available data for density of plastic polymers.

While this set of simulations does not provide a full evaluation of the sensitivity matrix for these parameters since we varied only one at a time and an actual scenario might simultaneously vary all three, the simulations do impart appreciation for potential variability and thus, impact on inferences. The simulations for the rat studies allow evaluation of whether overload would be achieved or not with a specific exposure (*i.e.*, concentration, regimen / duration, MMAD, GSD and density) to inform inferences based on observed toxicity, and are also the basis for the simulations used to calculate human equivalent concentrations (HECs), as discussed below.

For extrapolation of the predicted rat retained mass to an HEC, human simulations were conducted for adult males with a V_T of 0.992 L and a breathing frequency of 21 bpm, or with 1.364 L and 33 bpm. These ventilatory values are from the ICRP (1994) and represent ventilation associated with activity levels of either light exercise or heavy exercise for adult males. It should be noted that this combination of V_T and bpm for the light exercise ventilation input parameters are equivalent to the default minute ventilation value (V_E) used by EPA of 1.25 m^3/hr. An occupational exposure duration of 40 years was simulated for the human predictions of retained mass in the PU region.

The dose metric used to operationally derive the HEC is the PU retained mass (mg) normalized to the PU surface area (SA) in cm^2 according to the established US EPA methods (US EPA, 1994). The MPPD model estimates a human pulmonary surface area of 66.3 m^2 for an 80 kg adult male. Simulations are performed iteratively to arrive at a human equivalent exposure concentration (HEC) that achieves the same internal dose metric (PU mass / PU SA) in humans as was achieved in rats under the experimental conditions, in this case using the Muhle et al. (1990) conditions as described previously. As was shown in [REF _Ref46769100 \h * MERGEFORMAT], the predicted retained mass in the PU region corresponds well with the observed experimental data. The last two rows of the tables demonstrate the difference in HEC value due to variation in ventilatory parameters associated with either light or heavy activity. The human ventilation rate used in the simulations to calculate the HEC has direct impact on the relative contribution of deposition mechanisms and interacts with particle size especially as presented in [REF _Ref46770696 \h] and [REF _Ref46770706 \h]. [REF _Ref46770696 \h] shows the same simulation using an MMAD of 0.1 μm, whereas [REF _Ref46770706 \h] was run for a particle MMAD of 10.0 μm.

Table [SEQ Table * ARABIC]. MPPD predictions and HEC calculations for a hypothetical PVC exposure to F344 Rats with a particle MMAD of 0.1 μm, GSD of 2.07, and density of 1.3 gm / cm^3.

Exposure Concentration (mg/m^3)	3.3	8.3	20.2
Predicted Rat Retained PU Mass (mg)	2.65	8.43	23.3
Predicted Rat Retained PU Mass / PU SA (mg/m^2)	13.2	42.2	117
Light Activity 40-Year HEC (mg/m^3)	0.74	2.35	6.5
Heavy Activity 40-Year HEC (mg/m^3)	0.36	1.14	3.16

HEC = human equivalent concentration that results in the same inhaled dose metric (retained PU mass / PU SA) as predicted for the rat. The human ventilatory parameters of the light and heavy activity levels for

simulation of 40-year occupational scenario are described in the text.

Table [SEQ Table * ARABIC]. MPPD predictions and HEC calculations for a hypothetical PVC exposure to F344 Rats with a particle MMAD of 10 μm, GSD of 2.07, and density of 1.3 gm / cm^3.

Exposure Concentration (mg/m^3)	3.3	8.3	20.2
Predicted Rat Retained PU Mass (mg)	0.024	0.073	0.23
Predicted Rat Retained PU Mass / PU SA (mg/m^2)	0.12	0.36	1.15
Light Activity 40-Year HEC (mg/m^3)	0.058	0.177	0.560
Heavy Activity 40-Year HEC (mg/m^3)	0.011	0.034	0.109

HEC = human equivalent concentration that results in the same inhaled dose metric (retained PU mass / PU SA) as predicted for the rat. The human ventilatory parameters of the light and heavy activity levels for simulation of 40-year occupational scenario are described in the text.

Message

From: Becker, Rick [Rick_Becker@americanchemistry.com]
Sent: 7/27/2020 5:53:02 PM
To: Sahar_Osman-Sypher@americanchemistry.com; Hayes, Michael [hayes.mp@pg.com]; Hillebold, Donna [donna.hillebold@nouryon.com]; ljovanovich@stepan.com; Keene, Athena M. [Athena.Keene@AftonChemical.com]; Kennedy, Wayne [wayne.kennedy@aftonchemical.com]; Moors, Stefan [stefan.moors@basf.com]; Ogden, Julianne [Julianne_Ogden@americanchemistry.com]; Skulsky, Joseph [JSkulsky@stepan.com]; Tveit, Ann [Ann.Tveit@basf.com]; Washburn, Kenneth [Kenneth.Washburn@us.sasol.com]; Yang, Xinyu [xyang@Solenis.com]; Stedeford, Todd [Stedeford.Todd@epa.gov]; Henry, Tala [Henry.Tala@epa.gov]; Salazar, Keith [Salazar.Keith@epa.gov]; Irwin, William [Irwin.William@epa.gov]
Subject: attached -General Surfactants Manuscript Draft - July 23 Version 4 with a few edits from today's call in track changes
Attachments: draft manscript general surfactants - 23 July 2020.ver.4+ Becker.docx

All - thanks again for a productive call. Attached is the General Surfactants Manuscript Draft - July 23 Version 4 with a few edits from today's call -- all edits are in track changes.

Best

Rick

Richard A. Becker Ph.D. DABT | American Chemistry Council
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From: Osman-Sypher, Sahar
Sent: Friday, July 24, 2020 6:08 PM
To: Becker, Rick; Hayes, Michael; Hillebold, Donna; Jovanovich, Lela; Keene, Athena M.; Kennedy, Wayne; Moors, Stefan; Ogden, Julianne; Skulsky, Joseph; Tveit, Ann; Washburn, Kenneth
Subject: RE: General Surfactants Manuscript Draft - July 23 Version 4
Importance: High

All: I did not receive any comments from the workgroup members on the version I circulated today (attached – July 23, Version 4). I need our workgroup toxicologists to spend some time to review t Ex. 5 Deliberative Process (DP) and be prepared to share your thoughts on Monday's call. Thanks in advance.

Sahar

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From: Osman-Sypher, Sahar
Sent: Friday, July 24, 2020 8:44 AM
To: Becker, Rick <Rick_Becker@americanchemistry.com>; Hayes, Michael <hayes.mp@pg.com>; Hillebold, Donna <donna.hillebold@nouryon.com>; Jovanovich, Lela <ljovanovich@stepan.com>; Keene, Athena M. <Athena.Keene@AftonChemical.com>; Kennedy, Wayne <wayne.kennedy@aftonchemical.com>; Moors, Stefan <stefan.moors@basf.com>; Ogden, Julianne <Julianne_Ogden@americanchemistry.com>; Skulsky, Joseph <JSkulsky@stepan.com>; Tveit, Ann <Ann.Tveit@basf.com>; Washburn, Kenneth <Kenneth.Washburn@us.sasol.com>
Subject: General Surfactants Manuscript Draft - July 23 Version 4 | Comments Due by 2 pm Eastern Today
Importance: High

To: General Surfactants Category Workgroup

Attached is the latest version of the manuscript (July 23, Version 4). This includes edits from Todd Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP) He thinks this still needs work but wanted to share to solicit feedback from the team. ***Please review and send me any further edits by 2 pm Eastern today*** so I can include into the version for discussion on our next call with EPA on Monday. It would be helpful if you can flag what pages or sections you made changes to so I can be sure your edits are captured into the next version.

Rick – I've incorporated your edits from yesterday to address ScitoVation's comments into this version.

Mike/Wayne – I know you are working on Ex. 5 Deliberative Process (DP) so I expect we can send a new version of that to the team later today as well.

We have our call with EPA scheduled for **Monday, July 27th, 12-1:30 pm Eastern**.

Regards, Sahar

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Surfactants Category: The Application of New Approach Methodologies (NAMs) for Assessing Inhalation Risks under the Amended Toxic Substances Control Act

Commented [HT1]: Should intro have a bit more related to exposure? And how to fit in the irritation/corrosion properties of surfactants relative to inhalation?

Tala R. Henry[a,‡], *Keith Salazar*[b,‡], *Michael P. Hayes*[c], *Wayne Kennedy*[d], *Athena M. Keene*[d], *Annie Jarabek*[e], *Stefan Moors*[f], *Lela Jovanovich*[g], *Raphael Tremblay*[c], *Ann Tveit*[f], *Richard A. Becker*[h], *Sahar Osman-Sypher*[h], *Patrick D. McMullen*[i], *Scott D. Slattery*[i], *William Irwin*[b], *Marc Odin*[j], *Julie Melia*[j], *and Todd Stedeford*[a,*]

[a] Office of Pollution Prevention and Toxics, Office of Chemical Safety and Pollution Prevention, U.S. Environmental Protection Agency, Washington, DC 20460, United States

[b] Risk Assessment Division, Office of Pollution Prevention and Toxics, Office of Chemical Safety and Pollution Prevention, U.S. Environmental Protection Agency, Washington, DC 20460, United States

[c] Proctor & Gamble, Company, Inc., St. Bernard, Ohio 45217, Untied States; Temselaan 100, 1853 Strombeek-Beaver, Belgium

[d] Afton Chemical Corporation, Richmond, Virginia 23219, United States

[e] Health & Environmental Effects Assessment Division, Center for Public Health & Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina 27711, United States

[f] BASF Personal Care and Nutrition GmbH, GBP/RD, Gebäude Z22, Henkelstrasse 67, 40589 Duesseldorf, Germany; BASF Corporation, Florham Park, New Jersey 07932, United States

[g] Stepan Company, Northfield, Illinois 60093, United States

[h] American Chemistry Council, Washington, DC 20002, United States

[i] ScitoVation, Durham, North Carolina 27713, United States

[j] SRC, North Syracuse, New York 13212, United States

KEYWORDS (Word Style "BG_Keywords"). If you are submitting your paper to a journal that requires keywords, provide significant keywords to aid the reader in literature retrieval.

ABSTRACT

[To be added after co-authors feedback] The abstract should briefly state the problem or purpose of the research, indicate the theoretical or experimental plan used, summarize the principal findings, and point out major conclusions. Abstract length is one paragraph.

INTRODUCTION

The Toxic Substances Control Act (TSCA) is the primary chemicals management law in the United States and was enacted to ensure the protection of health and the environment against unreasonable risks of injury from chemical substances. In 2016, the Frank R. Lautenberg Chemical Safety for the 21st Century Act (Pub. L. 114-182; hereinafter the "Lautenberg amendments") was signed into law, thereby amending TSCA. The Lautenberg amendments included substantial changes to EPA's

authorities and responsibilities under TSCA, including requirements on EPA to make determinations on new chemical substances for unreasonable risk, sufficiency of information with determining risk, and exposure-based risk determinations. The amended TSCA also included provisions mandating the reduction and replacement of vertebrate animals in testing, to the extent practicable and scientifically justified, in support of making a determination of unreasonable risk for new and existing chemical substances. TSCA section 4(h) also charges EPA with encouraging and facilitating:

> (1) the use of scientifically valid test methods and strategies that reduce or replace the use of vertebrate animals while providing information of equivalent or better scientific quality and relevance that will support regulatory decisions under TSCA;
> (2) the grouping of 2 or more chemical substances into scientifically appropriate categories in cases in which testing of a chemical substance would provide scientifically valid and useful information on other chemical substances in the category; and
> (3) the formation of industry consortia to jointly conduct testing to avoid unnecessary duplication of tests, provided that such consortia make all information from such testing available to the Administrator.

The present investigation advances each of these TSCA mandates for chemical substances characterized as surfactants.

A surfactant is a substance that reduces the surface tension of a liquid in which it is dissolved. They are surface-active, amphiphilic compounds that self-assemble to form micelles or aggregates above a critical concentration, referred to as the critical micelle concentration (CMC). These substances are commonly used in occupational settings, in consumer products (*e.g.*,

household cleaning products, personal care products, *etc*.), and in biological research and development (R&D) as detergents, wetting agents, emulsifiers, foaming agents, and dispersants. Their use in such applications provide pathways of exposure by which potential toxicity of these compounds may occur to human or environmental receptors. Specifically, the inherent properties of surfactants may induce toxicity if exposures occur such that they can interfere with biological surfactants or tissues. For example, sodium dodecyl sulfate, a strong anionic surfactant, is used in R&D applications at concentrations up to 10% to disrupt cell membranes and to denature proteins, whereas octylphenoxypolyethoxyethanol, a mild nonionic surfactant, is used in R&D applications up to 1% to disrupt cell membranes, while preserving proteins for isolation (Burden, 2012).

Hazard concerns for surfactants were historically focused on their observed environmental effects and potential toxicity to aquatic organisms (Cowan-Ellsberry, 2014). For example, the U.S. Environmental Protection Agency (EPA) established chemical categories for cationic (quaternary ammonium) and anionic surfactants based on environmental toxicity concerns (EPA, 2010). Surfactants may also be a potential hazard concern to humans, depending on the use and route of exposure, because they can disrupt the normal architecture of the lipid bilayer and reduce the surface tension, thereby solubilizing cell membranes. For example, mucous membranes are particularly sensitive to the surface-active effects of surfactants, which have been shown to cause irritancy and injury to the eye, based on their ability to "readily penetrate the sandwiched aqueous and lipid barriers of the cornea" (Fox and Boyes, 2008).

Depending on the conditions of use, inhalation exposures to workers and/or consumers may be possible that warrant consideration in quantitative risk assessments. As noted, surfactants may cause adverse effects on mucous membranes, including the respiratory tract, and have been shown to interfere with the natural pulmonary surfactants, resulting in reduced oxygen content of arterial blood (*i.e.*, impaired gas exchange in the lung), increases in pulmonary extravascular water volume and wet-to-dry weight ratio of the lungs, grossly visible pulmonary edema, and atelectasis (Nieman and Bredenberg, 1985; Wang et al., 1993; Modell et al., 1969). However, the chemical space for surfactants that may present inhalation hazards has not been previously defined, and the potential for inhalation toxicity ranges by orders of magnitude, such as Octoxynol 9, a nonionic surfactant (Triton-X 100; CASRN 9002-93-1; 14-day lowest-observed-adverse-effect concentration [LOAEC] of 5.3 mg/m^3) (EPA, 2016; ECHA, 2020), versus didecyldimethyl ammonium chloride, a cationic surfactant and biocide (DDAC, CASRN 7173-51-5; 4-week lowest-observed-adverse-effect concentration [LOAEC] of 0.08 mg/m^3 for portal-of-entry effects) (MDEQ, 2003; CIR, 2003; ECHA, 2020).

The purpose of the present investigation was to: (1) perform a systematic review of the literature with the aim of defining the chemical space for surfactants; (2) identify appropriate toxicological analogues, when available, for identifying potential inhalation hazards and when data allow, identifying quantitative point(s) of departure for use in an inhalation risk assessment; (3) describe scientifically sound new approach methodologies (NAMs) to reduce or replace animal testing, where possible; and (4) establish a tiered-testing strategy, that utilizes NAMs, as appropriate, for new chemistries in the surfactant space.

MATERIALS AND METHODS

Systematic Literature Review

Commented [OS2]: Todd to summarize and move the details to an appendix

Objective

The objective of the literature search, screening, and retrieval process was to obtain studies that evaluated the toxicity of surfactants in the lower respiratory tract (LRT or thoracic region; *i.e.*, tracheobronchial and pulmonary regions) in exposed humans, investigated LRT outcomes in laboratory animals, or informed an adverse outcome pathway or mode of action for these agents at a cellular level (*i.e.*, *in vitro* studies). Because a list of surfactants with Chemical Abstracts Service Registry Numbers (CASRNs) was not known *a priori*, the initial PubMed search strategy was broad, with the intention of capturing potentially relevant information on any surfactant compound. Additional search strategies were employed to obtain studies not identified by keyword searching using Medical Subject Headings (MeSH or mh) and text words (tw) in PubMed.

PubMed Search

Computerized literature searches were initially conducted in PubMed in November 2016 to obtain studies related to the toxicity of surfactants in the LRT of humans and experimental animals. The search query string is presented in Table 1.

Table 1. PubMed search strategy for lung effects of surfactants.

Database Search Date	**Query String**[a]
PubMed 11/15/2016	("surface-active agents"[mh] AND lung[mh]) AND ((detergents[mh] OR aerosols[mh] OR "pulmonary surfactants"[mh]) OR (lung diseases[mh] OR cell respiration[mh] OR surface tension[mh]))

[a] Note, an Updated Literature Search was performed in April 2018, which excluded an expanded list of MeSH, query, and text words. Further details are provided in the Supplemental Information file titled "[redacted]".

Screening methods for this search included manual screening of titles/abstracts and screening of full text articles using the PECO criteria shown in Table 2.

Table 2. PECO criteria for screening of literature search results for lung effects of surfactants.

PECO element	**Evidence**[a]
Population	Humans, laboratory animals (rats, mice, hamsters, guinea pigs, dogs, non-human primates, or other inbred mammals) and mammalian cell lines
Exposure	*In vivo* (all routes), *ex vivo* (isolated perfused lung), and *in vitro*
Comparison	Any comparison (across dose, duration, or route) or no comparison (*e.g.*, case reports without controls)
Outcomes	Any examination of: • Pulmonary effects *in vivo* or *ex vivo* studies • Cytotoxicity or alternative methods in *in vitro* studies

[a] The PECO criteria were refined and more specific in the Updated Literature Search performed in April 2018. For more details, see the Supplemental Information file titled "[redacted]".

Additional Search Strategies (Gray Literature, Tree Searching, and Literature Search)

A search of the gray literature[1] was performed in September 2018 to obtain additional information pertaining to lung effects of surfactants. Resources searched for pertinent gray literature are listed in Table 3. The chemicals and compound groups identified from the initial literature search and used for gray literature searching are listed in Table 4. Screening methods for this search included manual screening of titles/abstracts and full text reports using the PECO criteria shown above in Table 2.

Table 3. List of resources to search for gray literature.

ATSDR [HYPERLINK "http://www.atsdr.cdc.gov/toxprofiles/index.asp"]
Chemtrack [HYPERLINK "http://www.chemtrack.org/White/CMR.pdf"]
CIR [HYPERLINK "http://www.cir-safety.org/ingredients"]
ECETOC publications [HYPERLINK "http://www.ecetoc.org/publications"]
ECHA [HYPERLINK "http://echa.europa.eu/web/guest/information-on-chemicals/registered-substances"]
EFSA (European Food Safety Authority) [HYPERLINK "http://www.efsa.europa.eu/"]
EPA – ChemView (incl. TSCATS data) [HYPERLINK "https://chemview.epa.gov/chemview"]
EPA – HPV Hazard Characterization Documents [HYPERLINK "http://iaspub.epa.gov/oppthpv/hpv_hc_characterization.get_report?doctype=2"]

[1] Gray literature, as used herein, has the same meaning as defined by EPA (2018) and "refers to sources of scientific information that are not formally published and distributed in peer-reviewed journal articles. These references are still valuable and consulted in the TSCA risk evaluation process. Examples of gray literature are theses and dissertations, technical reports, guideline studies, conference proceedings, publicly-available industry reports, unpublished industry data, trade association resources, and government reports."

Table 3. List of resources to search for gray literature.

EPA – HPV Risk-Based Prioritization Documents (RBPs) [HYPERLINK "http://iaspub.epa.gov/oppthpv/hpv_hc_characterization.get_report?doctype=1"]
EPA – HPVIS via ChemID - [HYPERLINK "https://chem.nlm.nih.gov/chemidplus/chemidlite.jsp"]
EPA – TSCATS 1 (available via Toxline)
EPA – pesticides - [HYPERLINK "https://iaspub.epa.gov/apex/pesticides/f?p=CHEMICALSEARCH:1"] Archive [HYPERLINK "https://archive.epa.gov/pesticides/reregistration/web/html/status.html"]
FDA [HYPERLINK "https://www.fda.gov/default.htm"]
HERA [HYPERLINK "http://www.heraproject.com/RiskAssessment.cfm"]
HSDB [HYPERLINK "http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB"]
INCHEM (CICADS, EHC, HSG, IARC, IPCS, JECFA, SIDS) [HYPERLINK "http://www.inchem.org/"]
JECDB (Japan Existing Chemical Data Base) [HYPERLINK "http://dra4.nihs.go.jp/mhlw_data/jsp/SearchPageENG.jsp"]
NICNAS http://www.nicnas.gov.au/
NITE [HYPERLINK "http://www.safe.nite.go.jp/jcheck/search.action?request_locale=en"]
NTP [HYPERLINK "https://ntpsearch.niehs.nih.gov/home"]
OECD [HYPERLINK "http://www.echemportal.org/echemportal/page.action?pageID=9"]
OECD/SIDS [HYPERLINK "http://webnet.oecd.org/hpv/ui/SponsoredChemicals.aspx"]

Table 3. List of resources to search for gray literature.

ATSDR = Agency for Toxic Substances and Disease Registry; CICADS = Concise International Chemical Assessment Document; CIR = Cosmetic Ingredient Review; ECETOC = European Centre for Ecotoxicology and Toxicology of Chemicals; ECHA = European Chemicals Agency; EFSA = European Food Safety Authority; EHC = Environmental Health Criteria; EPA = Environmental Protection Agency; FDA = Food and Drug Administration; HERA = Human and Environmental Risk Assessment; HPV = High Production Volume; HPVIS = High Production Volume Information System; HSDB = Hazardous Substances Data Bank; HSG = Health and Safety Guideline; IARC = International Agency for Research on Cancer; INCHEM = Internationally Peer Reviewed Chemical Safety Information; IPCS = International Programme on Chemical Safety; JECDB = Japan Existing Chemical Data Base; JEFCA = Joint Expert Committee on Food Additives; NICNAS = National Industrial Chemicals Notification and Assessment Scheme; NITE = National Institute of Technology and Evaluation; NTP =National Toxicology Program; OECD = Organisation for Economic Cooperation and Development; SIDS = Screening Information Data Set; TSCATS = Toxic Substances Control Act Test Submissions

Table 4. Surfactants, constituent names, and CASRNs to use for searching gray literature.

Chemical Group or Constituent Name	**CASRN**
Alkoxysilane resins	Not applicable; chemical group term
Defomaire	No data
Alevaire OR tyloxapol	25301-02-4
Triton X-100 OR polyethylene glycol p-isooctylphenyl ether	9002-93-1
Dioctyl sodium sulfosuccinate (DOSS) or butanedioic acid, 2-sulfo-, 1,4-bis(2-ethylhexyl) ester, sodium salt (1:1)	577-11-7
Polyoxyethylene-10-oleyl ether (C18:1E10)	9004-98-2
Polyoxyethylene-10-dodecyl ether (C12E10)	6540-99-4
N,N-dimethyl-dodecylamine-N-oxide (C12AO)	1643-20-5

The reference lists of the primary studies and review articles identified by the PubMed search were manually screened to identify additional pertinent literature for lung effects of surfactants (*i.e.*, tree searching). An Updated Literature Search was performed in April 2018. The details of

this search are provided in the Supplemental Information file titled "___". This literature search was used to identify additional studies or data related to LRT effects of surfactants that became available after the original search was conducted.

Risk Assessment Approaches under TSCA

Risk Assessment Paradigm

The current methods and approaches of risk assessment, both across EPA and as articulated in TSCA, have been built upon decades of expert development, scientific peer review, refinement, and scientific knowledge. Generally, EPA conducts risk assessments following the four-step process articulated by the National Research Council in 1983 (NRC, 1983) and reaffirmed as an appropriate approach several times since (NRC, 1994; NRC, 2009). This process includes hazard identification, dose-response analysis, exposure assessment, and risk characterization. Hazard assessment (also called effects assessment in some EPA guidance documents) identifies the types of adverse health or environmental effects or hazards that can be caused by exposure to the chemical substance in question and characterizes the quality and weight of scientific evidence supporting this identification. In the dose-response assessment, the relationship between the exposure or dose of a chemical and the occurrence of health or environmental effects or outcomes is assessed. The exposure assessment characterizes the extent of human or environmental exposures, including the magnitude, frequency, and duration of the exposure, to the extent necessary and practicable within the context of the assessment. Finally, the risk characterization integrates the hazard, dose-response, and exposure assessment to describe the nature, and when possible, the magnitude of risks to human health and the environment.

The approaches employed for these components, including, for example, the level of detail and complexity of quantitative aspects may vary across different risk assessments and typically align with specific legislative and regulatory frameworks. For example, legislative and regulatory frameworks for hazard evaluation of pesticide active ingredients, anti-microbial substances, inerts, *etc*. are described in regulations for pesticides, which include multiple and specific requirements for toxicity data. Under TSCA and its implementing regulations (see EPA's Review Process for New Chemicals, 2020), companies are required to submit a Premanufacture Notice (PMN) along with all available data on: chemical identity, production volume, byproducts, use, environmental release, disposal practices, and human exposure. These submissions are required to include all existing health and environmental data in the possession or control of the submitter, parent company, or affiliates, and a description of any existing data known to or reasonably ascertainable by the submitter. However, TSCA has never included requirements for toxicity testing or generation of hazard data for new chemical substances prior to submission for review by EPA.

Commented [RAB3]: https://www.epa.gov/reviewing-new-chemicals-under-toxic-substances-control-act-tsca/epas-review-process-new-chemicals

Hazard Assessment

Given the lack of toxicity testing requirements under TSCA, EPA only occasionally receives empirical hazard data for new chemical substances. EPA recently conducted an analysis of toxicity tests submitted to EPA for new chemical substances under TSCA and found that ___% of PMN submissions included any type of toxicity testing and most were for aquatic toxicity. TSCA provides EPA with the authority to require generation and submission of additional data when the information included with the PMN, coupled with that available to EPA risk assessors from prediction modeling, read-across, internal archives, *etc*. is insufficient to permit a reasoned

Commented [HT4]: Website name; DIFFERENT THAN NAME OF DOCUMENT, which is really looong.

evaluation of the health and environmental effects of a new chemical substance. However, prior to making a request for testing using vertebrate animals, EPA must take into consideration reasonably available existing information, including toxicity information; computational toxicology and bioinformatics; and high-throughput screening methods and the prediction models of those methods (TSCA Section 4(h)(A)(i)-(iii)).

Given the historical lack of hazard data and the new requirements to consider reasonably available existing information, EPA has, for decades, relied on a number of approaches that do not rely on *de novo* toxicity testing, including computational toxicology (*e.g.*, predictive models and expert systems), analogue read-across (wherein available toxicity data for a chemical of similar structure and activity is used to assess the new chemical substance lacking data), and chemical categories (a group of chemicals whose properties are likely to be similar or follow a regular pattern as a result of mechanism, mode of toxic action or structural similarity) (van Leeuwan et al., 2009).

Commented [HT5]: van Leeuwen, K., Schultz, T.W., Henry, T., Diderich, B., Veith, G. 2008. Using chemical categories to fill data gaps in hazard assessment. *SAR and QSAR in Environ Res*, 20:207-220.

1.Dellarco, V., Henry, T., Sayre, P., Seed, J., Bradbury, S. 2010. Meeting the common needs of a more effective and efficient testing and assessment paradigm for chemical risk management. *J Toxicol Environ Health*, 13:347-360.

Dose-Response Analysis

For assessing hazards to human health, EPA relies most heavily on read-across methods using an analogue or a category of analogues to identify hazards and conduct dose-response analysis to identify a point of departure (POD). While EPA has a number of existing "TSCA New Chemicals Program (NCP) Chemical Categories" (EPA, 2010), including for anionic, nonionic, and cationic surfactants, the existing surfactant categories were developed and defined based only on environmental toxicity considerations. Toxicity tests for analogues are used to identify a point of departure (POD) (*i.e.*, a dose or concentration that marks the beginning of a low-dose

Commented [HT6]: EPA, 2020. TSCA New Chemicals Program (NCP) Chemical Categories. Office of Pollution Prevention and Toxics, Washington, DC.

[HYPERLINK "https://www.epa.gov/sites/production/files/2014-10/documents/ncp_chemical_categories_august_2010_version_0.pdf"]

Anionic Surfactants pg. 34//Eco only

Cationic (quaternary ammonium) Surfactants pg. 51//Eco Only

Nonionic Surfactants pg. 94//Eco only

extrapolation) for assessing risks to the new chemical substance. This point can be the lower bound on dose for an estimated incidence or a change in response level from a dose-response model (*i.e.*, benchmark concentration or dose [BM(C)D], NOAE(C)L, LOAE(C)L, or human equivalent concentration or dose [HE(C)D]) for an observed incidence or change in level of response) (EPA, 2017).

Once suitable analogues are identified, the strengths, limitations, and uncertainties associated with using the analogue as predictive of hazards of the new chemical substance are considered to derive a benchmark margin of exposure (MOE). The benchmark MOE is the result of multiplying all relevant uncertainty factors (UFs) to account for: (1) the variation in susceptibility among the members of the human population (*i.e.*, inter- individual or intraspecies variability); (2) the extrapolation from animal data to humans (*i.e.*, interspecies extrapolation); (3) the extrapolation from data in a study with less- than- lifetime exposure (*i.e.*, extrapolating from sub-chronic to chronic exposure); (4) the extrapolation from a LOAEL rather than from a NOAEL; and (5) the potential derivation of an under-protective value as a result of an incomplete characterization of the chemical's toxicity (EPA, 2002, 2011). EPA prefers using existing information to set the magnitude of the UF value (EPA, 2014). However, data-derived UFs (known as data derived extrapolation factors – DDEFs or chemical specific adjustment factors – CSAFs) are not often possible, especially for new chemical substance, thereby requiring the use of default UFs.

Exposure Assessment

Commented [HT7]: RfD/RfC Guidance has a really nice figure showing the duration and DAF adjustments… include??

In assessing new chemical substances, EPA typically generates the human exposure estimates for workers using modeling approaches including the Chemical Screening Tool for Exposures and Environmental Releases (ChemSTEER). ChemSTEER exposure estimates are generated as daily

acute potential dose rates (PDRs) in mg/kg-bw/day or lifetime average daily doses (LADDs) in mg/kg-bw/day. Given that new chemical substances will not have occupational exposure monitoring data, except for possible monitoring data on analogues, the PDR is typically used as an initial conservative exposure estimate when calculating the MOE.

Due to the surface-activity of surfactants at the point of exposure, the PDR is the appropriate dose-metric. For chemical substances used in a liquid, mist, or aerosol form, the general default PDR value is 1.875 mg/kg-bw/day (*i.e.*, 15 mg/m^3; 1.875 mg/kg-bw/day × 80 kg-bw ÷ 10 m^3/day) (EPA, 2013 [ChemSTEER manual]). A summary of the default values used for calculating PDRs for new chemical substances in mist or aerosol form is provided in Table 6.

Table 6. Default values used for calculating the PDR.

Description	Equation	Description	Equation[a]	Defaults	Units
PDR (mg/kg-bw/day)	I/BW	Inhalation PDR (I)	Cm × b × h, where Cm is the mass concentration of chemical in air, b is the volumetric inhalation rate ($0 < b \leq 7.9$), and h is the exposure duration ($0 \leq h \leq 24$)	Cm = 15 mg/m^3 b = 1.25 m^3/hr h = 8 hours/day	mg/day
		Body weight (BW)	BW ($0 \leq BW$)	80 kg	Kg

[a] Cm may also be adjusted for the mass concentration of the chemical with a PEL in air (Based on OSHA PEL – TWA; default = 15 mg/m^3), the weight fraction of chemical in particulate(Ys) ($0 < Ys \leq 1$), the weight fraction of chemical or metal with a PEL in particulate (Ypel) ($0 < Ypel \leq 1$) using the following equation: Cm = KCk × Ys/Ypel

Occupational exposures are most often reported as 8-hr TWAs for exposures during workdays (5 days/week) and therefore, discontinuous exposures of animal studies are adjusted to derive HECs relevant to the occupationally exposed human population. The optimal approach is to use a physiologically-based pharmacokinetic model; however, the data required to conduct such modelling rarely exist for new chemical substances. Therefore, occupational exposures are adjusted using particle deposition models with human exertion (work) ventilation rates and exposure durations appropriate to the particular occupational setting and chemical use scenario. A duration adjustment is applied to the POD to account for the exposure conditions under evaluation (*e.g.*, workers = 8 hours/day, 5 days/week) versus the exposure conditions employed in the experimental study (*e.g.*, 6 hours/day, 5 days/week).

Commented [HT8]: (U.S. EPA, 1994).

Risk Characterization

Risk characterization is an integral component of the risk assessment process for both ecological and health risks, *i.e.*, it is the final, integrative step of risk assessment. As defined in EPA's Risk Characterization Policy, the risk characterization integrates information from the preceding components of the risk assessment and synthesizes an overall conclusion about risk that is complete, informative, and useful for decision makers. In essence, a risk characterization conveys the risk assessor's judgment as to the nature and existence of (or lack of) human health or ecological risks (EPA, 2000). As noted in EPA's Risk Characterization Handbook "Risk characterization at EPA assumes different levels of complexity depending on the nature of the risk assessment being characterized. The level of information contained in each risk

characterization varies according to the type of assessment for which the characterization is written and the audience for which the characterization is intended."

Risk characterization is performed by combining the exposure and dose-response assessments. Under TSCA section 5, EPA must determine whether a chemical substance presents an unreasonable risk of injury to health or the environment under the conditions of use. EPA generally uses an MOE approach to characterize risks of new chemical substances as a starting point to estimate non-cancer risks for acute and chronic exposures. The MOE is the HEC derived from a POD for a specific health endpoint (from hazard assessment) divided by the exposure concentration for the specific scenario of concern (from exposure assessment). To determine whether the resulting MOE results in an adequate margin between human exposure estimates and the HEC derived from a POD, the MOE value is compared with a pre-determined benchmark MOE. When using MOEs as risk estimates for non-cancer health effects, the benchmark MOEs are used to interpret the risk estimates. Human health risks are interpreted when the MOE is less than the benchmark MOE. On the other hand, negligible concerns would be expected if the MOE exceeds the benchmark MOE. Typically, larger MOEs (if greater than the benchmark MOE) result in a lower likelihood that a non- cancer adverse effect will occur. MOEs allow for providing a non-cancer risk profile by presenting a range of estimates for different non-cancer health effects for different exposure scenarios and are a widely recognized point estimate method for evaluating a range of potential non-cancer health risks from exposure to a chemical.

In summary, to conduct a risk evaluation for new chemical substances, as required under TSCA section 5, EPA conducts a hazard assessment, using empirical data when available, but most

often using analogues, to identify a POD(s) and to develop a benchmark MOE that reflects specific uncertainties associated with data available for use in the evaluation. This hazard assessment is combined with the exposure assessment, to calculate an MOE, which is compared to the benchmark MOE to determine whether risks are identified. The risk characterization is used to inform the "unreasonable risk" determination.

RESULTS AND DISCUSSION

Literature Search and Screening Results

The results of the literature search and screening effort are presented graphically in Scheme 1. The PubMed search identified 43 potentially relevant studies for full text review. The PubMed search results were supplemented by a search of gray literature resources, which identified six references for full text review. The Updated Literature Search identified nine additional studies for full text review.

The full text review of 60 references yielded X potentially relevant studies with data on lung effects of surfactants (*i.e.*, references that were cited in this white paper). Studies that were excluded following full text review included X papers on compounds that were not used as surfactants. Studies were also excluded if they did not evaluate lung effects (n = X; no evaluation of respiratory function and/or pathological examination of the lungs).

Commented [ST9]: This section needs updating following final disposition of gray lit and Updated Literature Search.

Scheme 1. Literature search and screening flow diagram for surfactants

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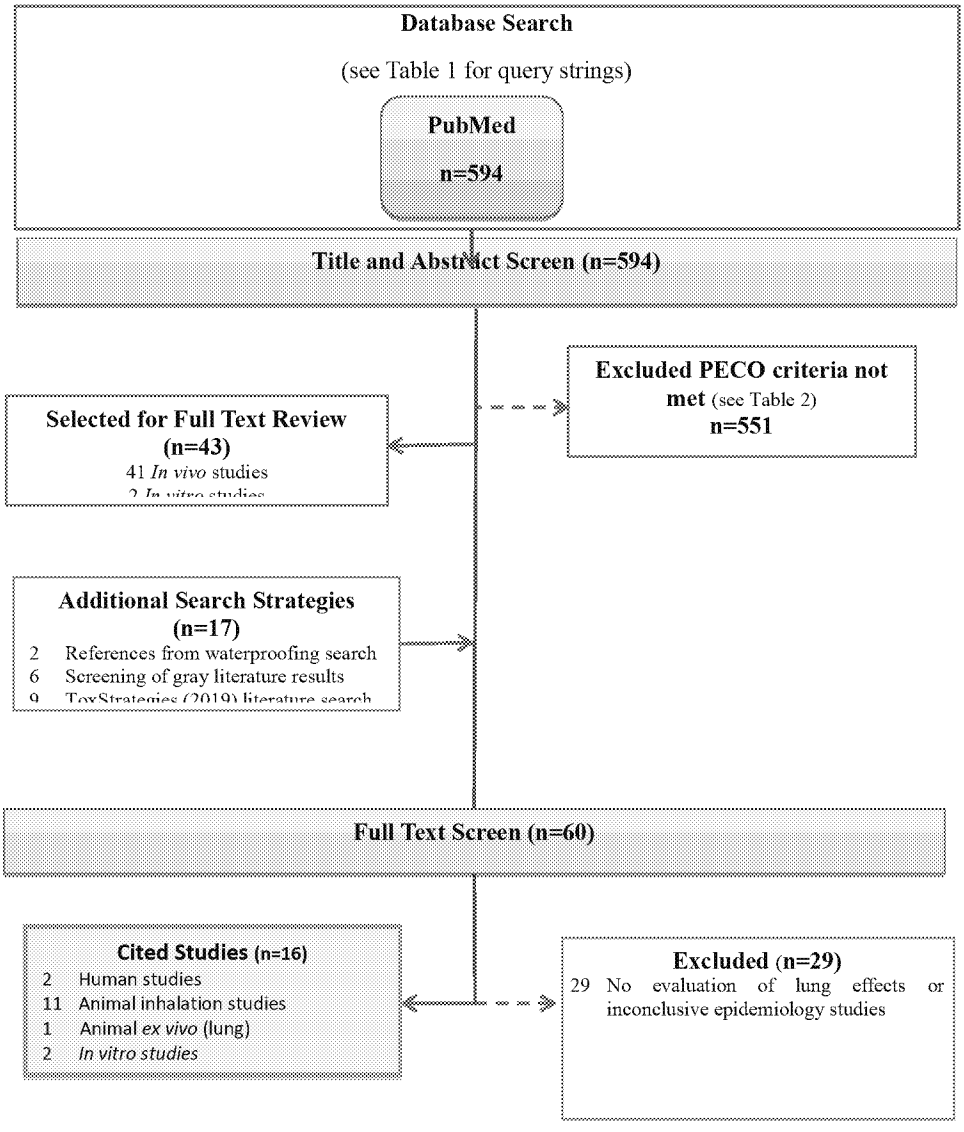

[PAGE]

Category Boundaries

Surfactants are comprised of three general subcategories including nonionic, anionic, and cationic substances. Within these subcategories, the following defined structural and functional criteria (hereinafter referred to as the "Surfactant Criteria") are used to distinguish chemical substances, which include polymers and UVCB substances,[2] intended for use as surfactants from other amphiphilic compounds (*e.g.*, ethanol) (EC, 2009, 2011; HTS, 2017):

1. A substance which has surface-active properties, and which consists of one or more hydrophilic and one or more hydrophobic groups;
2. The substance must be capable of reducing the surface tension between air and water to 45 milliNewtons/meter (mN/m) or below at a test condition of 0.5 wt% in water and a temperature of 20°C (*Cf.* Pure water has a surface tension of 72.8 mN/m at 20°C); and
3. The substance self-associates in water to form micellar or vesicular aggregates at a concentration of 0.5 wt% or below.

The Surfactant Categories were subcategorized for those chemical substances that initially meet the Surfactant Criteria and possess ionic or nonionic properties, as discussed below. Note, though not listed in the following subcategories, amphoteric chemical substances that meet the Surfactant Criteria would also be included within these subcategories (*i.e.*, cationic or anionic surfactants), depending on their pH. Lung lining fluids are near neutral pH, with various measurements ranging

[2] Chemical Substances of Unknown or Variable Composition, Complex Reaction Products and Biological Materials (UVCB Substance)

from 6.6 to 7.1 (Ng et al., 2004; Choudhary et al., Nielson et al., 1981). The pKa for each component of an amphoteric surfactant should be considered within this pH range and the assessment should be conducted on the predominant ~~or both~~ components. ~~The non-ionized fraction for acids/bases should be calculated as follows.~~

~~$\text{Acids Fraction}_{\text{Non-ionized}} = 1 / (1 + 10^{(pH - pKa)})$~~

~~$\text{Bases Fraction}_{\text{Non-ionized}} = 1 / (1 + 10^{(pKa + pH)})$~~

~~Where the pH represents the physiological pH in the lung (i.e., 6.6 to 7.1), and the pKa represents the value for the respective component (e.g., carboxylic acid or amine). A group has equal amounts of charged and neutral quantities at the pH value equal to the pKa value. At a pH value that is one unit below the pKa value, carboxyl groups are 10% negatively charged. At a pH value that is one unit above the pKa value, carboxyl groups are 90% negatively charged. At pH values below the pKa value, amine groups are positively charged. At a pH value that is one unit below the pKa value, amine groups are 90% positively charged. At a pH value that is one unit above the pKa value, amine groups are 10% positively charged. At physiological pH values, quaternary ammonium, phosphonium or sulfonium groups are positively charged while sulfonate and phosphonate groups are negatively charged.~~

Commented [KA11]: Should this sentence be deleted?

Commented [OS12]: Todd will update to simplify, show equation,
Aromatic amines is an aniline, ph7

Nonionic surfactants were identified as any neutral chemical substance that meets the Surfactant Criteria. Common nonionic surfactants include alkylphenol chemical substances with one or more than one ethoxylate (EO) unit as well as linear and branched alcohol chemical substances with one

or more EO units. Octoxyphenol with 9 EO units (CASRN 9002-93-1; a.k.a., octoxynol 9 or Triton-X 100), a common nonionic octylphenol EO surfactant and Polysorbate 80 or Tween 80 (CASRN 9005-65-6, another nonionic alkyphenol ethoxylate with increased alkyl chain length and number of EO units, are shown in Table X. The surface tensions of octoxynol 9, Polysorbate 20 and Polysorbate 80 have been reported as 30-31 mN/m at a concentration of 0.1% in water (33 mN/m, 1% actives at 25 °C) and 37.96 mN/m (0.5% at XX °C), respectively as shown in Table X (DOW, 2009, 2020; **Kothekar, et al., 2017**).

Commented [ST13]: Temp?

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Anionic surfactants were identified as any chemical substance with a net negative charge that meets the Surfactant Criteria (*e.g.*, alkyl sulfonates, alkylbenzene sulfonates, alkylether sulfates, alkyl silicic acids, alkyl phosphates, alkyl carboxylic acids, or combinations of these anionic groups). The structure of the common anionic surfactant SDS is shown in Table X. The surface tension of SDS is reported to be 39.5 mN/m at 25° C in water (Table X).

Commented [ST17]: Not in Mike's Table

Commented [ST18]: "The [HYPERLINK "https://en.wikipedia.org/wiki/Critical_micelle_concentration" \o "Critical micelle concentration"] (CMC) in pure water at 25 °C is 8.2 mM,[HYPERLINK "https://en.wikipedia.org/wiki/Sodium_dodecyl_sulfate" \l "cite_note-CMC-1"] and the [HYPERLINK "https://en.wikipedia.org/wiki/Aggregation_number" \o "Aggregation number"] at this concentration is usually considered to be about 62.[HYPERLINK "https://en.wikipedia.org/wiki/Sodium_dodecyl_sulfate" \l "cite_note-3"] The [HYPERLINK "https://en.wikipedia.org/wiki/Micelle" \o "Micelle"] ionization fraction (α) is around 0.3 (or 30%).[HYPERLINK "https://en.wikipedia.org/wiki/Sodium_dodecyl_sulfate" \l "cite_note-Barney_L-4"]"

[HYPERLINK "http://hera.ugr.es/doi/15008447.pdf"]
this paper shows ST to be a lot higher

Cationic surfactants were identified as any chemical substance with a net positive charge that meets the Surfactant Criteria (*e.g.*, alkylammonium chlorides and benzalkonium chlorides). The structure of the common cationic surfactant DDAC, as shown in Table X, is a representative member of this subcategory, although as noted previously, it also possesses biocidal properties. The surface tension of DDAC is reported to be 27.0 mN/m at 0.1% in water (Table X).

Commented [ST19]: Concentration?

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[INSERT TABLE X]

Hazard Identification

There is concern for dysfunction of natural surfactant in the lung from inhalation of surfactants. Additionally, there is evidence that some surfactants or similar structures may also interfere with the cell membrane (Jelinek et al., 1998, Parsi et al., 2015). The capacity of exogenous surfactants to interfere with pulmonary surfactant and impair pulmonary function has been demonstrated in human volunteers and in laboratory animals. The pulmonary response to surfactant aerosol is in proportion to the exposure concentration and duration, but available data are inadequate to identify effect levels, which in any case are likely to vary not only with the specific chemical surfactant, but also with the exposure method (*e.g.*, aerosol droplet size).

Commented [ST22]: Add the following, based on Updated Literature Search?
Evander et al. 1988
Rao & Das 1994
Ekelund et al. 2004

Note, exposure conditions need to be presented in the studies, e.g., 6 hrs/day, 5 days/week. Also, units should be consistently presented, e.g., mg/L versus mg/m3

Commented [OS23]: Parsi et al Phlebology. 2015 Jun;30(5):306-15. doi: 10.1177/0268355514534648.

In vitro toxicity of surfactants in U937 cells: cell membrane integrity and mitochondrial function
A Jelinek H P Klöcking Exp Toxicol Pathol. 1998 Sep;50(4-6):472-6.

Nonionic Surfactants

Several studies were found for the nonionic siliconized superinone respiratory detergent, formaldehyde, polymer with oxirane and 4-1,1,3,3-tetramethylbutylphenol (CASRN 25301-02-4; also known as Defomarie, Alevaire, Tyloxapol). Healthy human volunteers showed significantly decreased pulmonary compliance following acute inhalation of Defomaire beyond that produced by the distilled water control (Obenour et al., 1963). Increased minimum surface tension due to detergent was demonstrated, and shown to be dose-dependent, using pulmonary surfactant extracted from dogs and mixed *in vitro* with the nonionic surfactant tyloxapol (Alevaire) (Modell et al., 1969). *In vivo* exposure of dogs to Alevaire in this study (8 h aerosol exposure; vehicle and concentration not reported) produced little effect (only 1/10 dogs exposed to Alevaire showed

Commented [OS24]: Patrick McMullen Comment; Defomaire, Tyloxapol, Alevaire, and Superinone all refer to the same substance, correct? Recommend that after the first sentence it should be referred to using the same "name" each time.

increased minimum surface tension), which the authors concluded support the dose-dependence of the effect and indicate that small amounts of detergent can be present in the lungs without detectably altering surfactant function (Modell et al., 1969).

Other pulmonary effects in dogs and/or sheep exposed to nonionic surfactant, tyloxapol, included reduced oxygen content of arterial blood (*i.e.*, impaired gas exchange in the lung), increases in pulmonary extravascular water volume and wet-to-dry weight ratio of the lungs, and grossly visible pulmonary edema and atelectasis (*i.e.*, collapsed alveoli) (Nieman and Bredenberg, 1985; Wang et al., 1993; Modell et al., 1969). In the study by Modell et al., (1969), no gross pathology differences were seen in detergent-exposed vs. control lungs of dogs, although some portions of both control and exposed lungs were heavy and discolored reddish-purple, which may have been caused by fluid accumulation from the liquid aerosol exposures and/or the use of hypotonic saline in the study (0.45% NaCl). Normal appearances were observed in the remaining areas of the lungs.

In rodent models, irritation and inflammatory effects on the respiratory tract has been observed with varying degrees of severity. Acute inhalation exposure to Polysorbate 20 via nose-only administration for 4 hours in Wistar Han rats to a concentration of 5.1 mg/l (5,100 mg/m^3) did not observed in mortalities, clinical signs, or abnormalities in the gross pathology[3]. Using MPPD modeling, the total lung deposition mass was calculated to be 6.6E+4 µg. A respiratory irritation study was conducted on a mixture containing Nonidet in male Webster mice using the ASTM Method E981 where animals were exposed for 3 hours to concentrations of 12, 22, 51, 118, and

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[3] [HYPERLINK "https://echa.europa.eu/hr/registration-dossier/-/registered-dossier/13525/7/3/3"]

134 mg/m^3 (Alarie and Stock, 1992, unpublished). Signs of respiratory irritation was observed in animals at the three highest concentrations as indicated by increased respiratory frequency without an increase in pulmonary edema or lung weight. An acute inhalation exposure study in Syrian hamsters to 3.0 mg/l of Triton X-100 to varying exposure durations reported that lung deposition of Triton X-100 corresponded to mortality with an LD50 of 1300-2100 µg (Damon et al., 1982). The authors concluded that the deaths in these animals were likely the result of severe laryngeal edema and ulcerative laryngitis while the lower airways and lungs in these animals were relatively free of serious pathologies. The authors hypothesized that that these observed effects were due to large tracheobronchial deposition following the aerosol exposure and the mucociliary clearance of the deposited chemical resulted in a large concentration of the chemical on the laryngeal mucosa. Finally, in the only repeated dose inhalation exposure identified for nonionic surfactants, a 2-week repeated dose inhalation study was conducted on Triton X-100 in male and female Sprague-Dawley rats to 5.3 mg/m^3 (MMAD 1.8 µm, GSD 1.8µm) for 6 hours/day, 5 days/week (Bio/dynamics, Inc. 1992[4].) Slight to minimal subacute inflammation of the alveolar walls and hyperplasia of the alveolar/bronchiolar epithelium was reported, in addition to an increase in slight discoloration of the lungs, increased lung weight, and mucoid nasal discharge.

Commented [SK25]: It is unclear to me if the other tested concentration should be included since it is a 70% mixture.

In vitro studies of surfactant effects on cell membranes have provided evidence of possible MOAs. Warisnoicharoen et al., (2003) evaluated the cytotoxicity of the nonionic surfactants polyoxyethylene-10-oleyl ether ($C_{18:1}E_{10}$), polyoxyethylene-10-dodecyl ether ($C_{12}E_{10}$), and N,N-

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[4] Bio/dynamics, Inc. 1992. A two week inhalation toxicity study of C-437 and C-1754 (ethoxylated para-tertiary-octyl phenol) in the rat with cover letter dated 5/24/96 (sanitized). NTIS Report No. OTS0573048.

dimethyl-dodecylamine-N-oxide ($C_{12}AO$; CASRN 1643-20-5) to cultured human bronchial epithelium cells (16-HBE14o-) *in vitro*, using the MTT cell viability assay. All of the surfactants tested were cytotoxic at concentrations near or below their critical aggregation (micellular) concentrations (as determined by surface tension measurements), suggesting that surfactant toxicity was due to the disruption caused by the partitioning of monomeric surfactant into the cell membrane.

Lindenberg et al (2019) evaluated the cytotoxic activity of the of three nonionic polymeric surfactants, which are commonly used in formulations of nebulized pharmaceuticals to prevent protein agglomeration, Polysorbate 20 (Tween 20), Polysorbate 80 (Tween (80) and Poloxamer 188in a BEAS-2B human bronchial epithelial cell model by using an innovative air-liquid interface (ALI) method of exposure compared to classical liquid/liquid (L/L) model. The study measured the release of Lactate Dehydrogenase (LDH) which is an intercellular enzyme present in large amounts in the cytoplasm. Loss of membrane integrity will cause the release of LDH into the extracellular medium. Cytotoxicity of Polysorbate 20 was observed at concentrations of 1-2% (v/v) when using the more biologically relevant ALI method by measuring Lactate Dehydrogenase (LDH) activity, however, a significant increase in LDH was only observed at 4% for Polysorbate 80 and not significantly increased at concentrations of up to 10% for Poloxamer 188. These results suggest that Polysorbate 20 and to the lesser extent Polysorbate 80 induce damage to the cell membrane integrity while the linear Poloxamer 188 did not demonstrate any in vitro cytotoxicity.

Altogether, the available in vitro and in vivo data indicate a wide discrepancy in respiratory toxicity among nonionic surfactants. The small dataset presented in this section preclude establishing

correlations between respiratory effects and chemical properties such as surface tension or CMC. Others have examined the relationship between chemical properties of nonionic surfactants and eye irritation and concluded that hydrophilic-lipophilic balance, pH, alkyl chain length, or poly [oxyethylene] chain lengths failed to predict eye irritation potential across the nonionic subcategory (Heinze et al., 1999). However, significant correlations of eye irritation and the maximum reduction in surface tension were observed at the CMC or higher surfactant concentration when conducted under nonequilibrium conditions. Whether this chemical property similarly predicts potency of nonionic surfactants to induce respiratory effects requires additional data and analysis outside of the scope of this summary.

Anionic Surfactants

Two acute inhalation toxicity studies were identified for several anionic surfactants which demonstrated high toxicity via the inhalation route. Oleoyl sarcosine was evaluated in a 4-hour nose only inhalation study in male and female Sprague-Dawley rats using concentrations of 0.3, 0.6, 2.2, and 3.7 mg/L. An LC_{50} of 1.37 mg/L was identified with edema of the lung at 0.6 mg/L and audible gasping at 0.3 mg/L. For Sodium Lauroyl Sarcosinate (CASRN 137-16-6), 5 male Wistar rats were exposed to a 4-hour nose-only inhalation concentration of 0.05, 0.5, 1, and 5 mg/L and 5 female rats were exposed to 1.1 or 5.5 mg/L. All 10 animals exposed to 5 mg/L died within 1-2 h of dosing, and 4/5 of the animals exposed to 0.5 mg/L and the 10 animals exposed to 1 mg/ml died within 1-2 days after dosing. Animals in the 0.05 mg/l had no clinical signs or mortality at the conclusion of the study. At necropsy, red foci were noted on the lungs in animals of groups receiving concentrations of ≥ 0.5mg/L. The LC_{50} was reported to be 0.05-0.5 mg/L.

Commented [OS26]: Mike/Wayne have indicated that this does not meet the boundary criteria. It is quite insoluble, etc. More information to follow.

Commented [OS27R26]: William will address this in the table re: oleoyl sarcosine and sodium salt version.

Repeated-dose inhalation studies were identified for oleoyl sarcosine (CASRN 110-25-8), and dioctyl sodium sulfosuccinate (CASRN 577-11-7). Oleoyl sarcosine was evaluated in a 28-day nose-only inhalation study (OECD Guideline 412) in male and female Fischer rats (5/group/sex) using concentrations of 0, 0.006, 0.02, or 0.06 mg/L in 10% ethanol[5]. The mass median aerodynamic diameter (MMAD) of the aerosol particles were 1.11- 1.22 μm and the geometric standard deviation (GSD) was 1.68-2.57. Changes in the mean corpuscular volume (MCV), white blood cells (WBC), and lymphocytes in male animals of the high dose groups were observed. In female animals of the mid-dose group, reticulocyte counts were significantly reduced. Reflex bradypnea was noted in the animals of the mid and high doses which is associated with severely irritating substances. All test concentrations caused effects at several sites of the respiratory tract with indications for local irritation, such as squamous metaplasia and epithelium proliferation and submucous acute inflammation at the base of the epiglottis. In the lungs and bronchi, the most prominent finding was a focal early stage of fibrosis, but details were not provided at the dose level for this effect. Lung weights were increased at the highest dose. The NOEL was <0.006 mg/L (6 mg/m^3) air in males and females; the basis for the effect level was local irritation.

Commented [ST28]: Todd will add these footnotes (i.e., 3 + 4) to EndNote file

Dioctyl Sodium Sulfosuccinate was evaluated in a 13-week inhalation study in male and female Sprague-Dawley rats (12/group/sex), to an aerosol of a product containing of 4.2 mg/m^3, for 4 hours a day, 5 days a week[6]. There were no statistically significant differences in dosed and control

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[5] [HYPERLINK "https://echa.europa.eu/hr/registration-dossier/-/registered-dossier/21429/7/6/3"]

[6] Cosmetic, Toiletry, and Fragrance Association (CTFA). 1991. Acute oral, ocular, primary dermal irritation, 21-day dermal irritation, photocontact allergenicity,

groups, for the mean body weight gain, survival, appearance and behavior, urinalysis values, and microscopic lesions. Significant differences were noted in the blood such as elevated erythrocytic values in male rats at 7 weeks and depressed mean corpuscular hemoglobin concentration values in male rats at 13 weeks. At 7 weeks, the lungs of animals necropsied were stained with Oil Red O and examined; scattered foci of neutrophils and an increase in alveolar macrophages were reported in a single dosed male rat. A LOAEC of 4.2 mg/m^3 was identified based on blood effects in male rats.

Mechanistic studies examining the pulmonary effects of anionic surfactants have been studied in dogs and/or sheep exposed, dioctyl sulfosuccinate sodium salt. (DOSS; CASRN 577-11-7). Increased minimum surface tension of lung extract or bronchioalveolar lavage fluid (BALF) was observed in dogs and sheep following *in vivo* aerosol exposure to the anionic detergent dioctyl sodium sulfosuccinate (DOSS) in 1:1 mixture of ethanol and saline for 30 – 60 minutes, at a concentration that was selected to ensure a moderate degree of edema (estimated dose of 15 mg detergent/kg body weight) (Nieman and Bredenberg, 1985; Wang et al., 1993). Light microscopic examination of the lungs 4 hours after exposure to DOSS aerosol observed no grossly destructive effects on alveolar cells or lung architecture in exposed dogs. However, a decrease in pulmonary compliance was observed that the authors hypothesized was due to an increase in surface tension in the alveoli in the presence of detergent.

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6 RIPTs, 13-week subchronic dermal, 13-week subchronic inhalation, four 4-day mini-cumulative irritation. Submission of unpublished data by CTFA, 200 pp.

Pulmonary clearance studies using radiolabeled aerosol tracers have evaluated whether detergent effects on the surfactant layer lead to increased alveolar permeability. For example, inhalation exposure to DOSS enhanced the pulmonary clearance of radiolabeled diethylenetriamine pentaacetic acid (DTPA), a relatively small hydrophilic molecule, reflecting increased alveolar permeability after detergent exposure (Nieman et al., 1990; Nilsson and Wollmer, 1992, 1993; Evander et al., 1994; Tasker et al., 1996; Nilsson et al., 1997). In most studies, this effect on alveolar permeability was seen in the absence of effects on blood gas levels or pulmonary compliance that occur with higher exposure, indicating that the increase in alveolar permeability is a sensitive effect of detergent aerosol. The effect was demonstrated to be concentration-related in one study in which multiple dilutions of the liquid detergent were nebulized (Evander et al., 1994). Some studies also evaluated the clearance of a radiolabeled aerosol of albumin, a much larger molecule, which was enhanced by DOSS as well, but to a lesser degree than DTPA (Nilsson and Wollmer, 1992; John et al., 1997). Wang et al., (1993) observed an increase in protein flux from plasma to alveolar space after DOSS inhalation in sheep, which the authors attributed to disruption of the alveolar lining and increased microvascular permeability. The increased alveolar permeability observed in these studies has been hypothesized to result from increased alveolar surface tension, which could cause increased permeability either by opening previously closed pores (through which solutes pass) in the membrane or by stretching already open pores (Nieman et al., 1990; Wang et al., 1993). However, as previously mentioned, surfactants can disrupt cell membranes; thus, this mechanism may be an alternate explanation (Burden, 2012).

Cationic Surfactants

Acute Studies

Acute inhalation toxicity studies were identified for DDAC, Dioctadecyldimethylammonium chloride (DODMAC), and BAC. For DDAC, rats (5/sex/dose, unspecified strain) were exposed via inhalation to 0.05, 0.09, 0.13, 0.25, 1.36 mg/L, or 4.54 mg/L for 2 hours observed for 14 days. An LC_{50} of 0.07 mg/L was identified based on unspecified abnormalities identified in several organs including the lungs (EPA OPP RED). For DODMAC, Albino rats (10 males, strain not specified) were exposed to the test substance (1:29 distilled water) via inhalation at 180 mg/L for one hour and observed for 14 days (OECD SIDS, 1996). There were no mortalities. Treatment-related clinical signs included preening, excessive masticatory (chewing) movements, excessive salivation stains, lacrimation, serosanguineous stains around the nose and labored respiration. All animals appeared normal one day after dosing. The LD_{50} (1h) was > 180 mg/L. For BAC, female Wistar rats (5/group) were exposed via nose-only inhalation to 37.6 and 53 mg/m^3 for 4 hours and observed for 14 days or exposed to 30.6 mg/m^3 for 6 hours and BALF was measured 18 hours post-exposure (Swiercz et al., 2008). The identified LC_{50} was approximately 53 mg/m^3 and BALF analysis reported increased inflammatory markers such as TNF-a, IL-6 and an increase in indicators of lung damage such as LDH, total protein, and increased lung weight.

Repeated-Dose Studies

DDAC - didecyldimethyl ammonium chloride

Three repeated dose inhalation studies of three different exposure durations were identified for the cationic surfactant DDAC: 14-day, 20 to 21-day, and 90-day.

In the 14-day study, male Sprague-Dawley rats were exposed via whole-body inhalation exposures to DDAC aerosols of 0.15 mg/m^3, 0.6 mg/m^3, and 3.6 mg/m^3 (Lim et al., 2014). The

mass median aerodynamic diameter (MMAD) of the aerosols was 1.86 μm and the geometric standard deviation (GSD) was 2.75 μm. Mild effects were noted in the bronchoalveolar cell differentiation counts, cell damage parameters in the BAL fluids, in addition to inflammatory cell infiltration, and interstitial pneumonia of the medium and high groups. The NOAEC was determined to be 0.15 mg/m^3.

In the intermediate exposure study, male and female Sprague-Dawley rats (5 rats/sex/group) were exposed via dynamic nose-only inhalation for a total of 20 or 21 days to concentrations of 0, 0.08, 0.5, and 1.5 mg/m^3 (Weinberg, 2011). The MMAD was 1.4-1.9 μm and the GSD was 1.83-1.86 μm. Lung weights were increased in females in the mid- and high-concentration groups and in males in the high concentration group. The bronchoalveolar lavage fluid (BALF) analysis indicated that at the high concentration neutrophils and eosinophils increased with a concomitant decrease in macrophages. Ulceration of the nasal cavity was observed in males and females in the high concentration group. In males, there was an increase in cell count and total protein across all doses. In females, there was an increase in LDH across all concentrations, but the small sample size precluded establishing statistical significance for the effects. Minimal to mild increased mucus of the respiratory epithelium was observed in males and females at all concentrations. A conservative LOAEC of 0.08 mg/m^3 was identified based on increased mucus of the respiratory epithelium and increased LDH could be established for these effects; however, due to the mild effects and low number of animals/group, the effects were not statistically significant.

In the 13-week sub-chronic study, male and female Sprague-Dawley rats (10/group/sex) were exposed in whole body exposure chambers to concentrations of 0.11, 0.36, and 1.41 mg/m^3 (Kim et al., 2017). The MMAD of the DDAC aerosol was 0.63-1.65 µm, and the GSD was 1.62-1.65 µm. Body weight was confirmed to be clearly influenced by exposure to DDAC and mean body weight was approximately 35% lower in the high (1.41 ± 0.71 mg/m^3) male group and 15% lower in the high (1.41 ± 0.71 mg/m^3) female group compared to that of the control group. Albumin and lactate dehydrogenase were unaffected in the BALF. Lung weight was increased in females in the mid- and high-concentration groups in females and in males in the high concentration group only, which was accompanied by inflammatory cell infiltration and interstitial pneumonia in the mid- and high-concentration groups. Tidal volume and minute volume were not significantly affected at any concentration. Severe histopathological symptoms such as proteinosis and/or fibrosis, were not reported. A NOAEC of 0.11 mg/m^3 was identified based on the increased lung weights in females and increase in inflammatory cells.

BAC – benzalkonium chloride

BAC was evaluated in a 2-week whole-body inhalation study in male and female Fischer rats (5/group/sex) to concentrations 0.8, 4 and 20 mg/m^3 (Choi et al., 2020). The MMAD of the aerosols was 1.09-1.61 µm and the GSD was 1.51 to 2.00 µm. More exposure-related effects were observed in the upper airway. Nasal discharge, rale, and deep respiration were observed in the high dose group, and nasal discharge was observed in the low and mid dose groups. In the nasal cavity, ulceration with suppurative inflammation, squamous metaplasia, and erosion with necrosis were observed in the respiratory epithelium and transitional epithelium of the male and female high dose groups.

Degeneration and regeneration of terminal bronchiolar epithelium, smooth muscle hypertrophy of bronchioloalveolar junction, and cell debris in the alveolar lumens was observed in the mid and high dose male groups and high dose female group. Hypertrophy and hyperplasia of mucous cells in the bronchi or bronchiole were observed in both males and females. The authors hypothesized that BAC has greater deposition to the upper respiratory tract due to mucociliary clearance and emergency airway response caused by the irritation of BAC. The squamous metaplasia of the respiratory epithelium and transitional epithelium, mucinous cell hypertrophy and proliferation of the respiratory epithelium, mucinous cell metaplasia of the transitional epithelium in the nasal cavities, and mucinous cell hypertrophy and proliferation of terminal bronchiole which were observed in the study were considered adaptive changes after tissue injury. In the BALF analysis, the concentration of ROS/RNS, IL-1β, IL-6, and MIP-2 decreased dose dependently at the end of the exposure period but did not show a concentration-dependent change at 4 weeks of recovery. In addition, the concentrations of TNF-α, IL-4, and TGF-β did not show changes associated with test substance exposure. Finally, relative lung weights were statistically significantly increased in males at the mid and high doses and in females at the high doses only. The study authors concluded a LOAEC of <0.8 mg/ m^3 based on effects in the nasal cavity.

Mechanistic studies

Effects of cationic surfactant BAC on cell viability, inflammatory response and oxidative stress of human alveolar epithelial cells cultured in a dynamic culture condition were studied (Jeon, Haejun, et. al., 2019). To reflect the natural microenvironment of the lung, particularly its dynamic nature, the authors simulated normal breathing levels (tidal volume 10%, 0.2Hz) through surface

elongation of an elastic membrane in a dynamic culture system. This type of dynamic system provided easy control of breathing rate during lung cell culture. The system assessed the toxicity using different BAC concentrations (0, 2, 5, 10, 20, and 40 µg/mL) under static and dynamic culture conditions. Following 24 hr exposure to BAC, cellular metabolic activity, interleukin-8 (IL-8) and reactive oxygen species (ROS) levels demonstrated significant differences when using either static or dynamic cell growth conditions. The dynamic culture system, which more closely mimics lung conditions, showed higher toxic response to BAC.

Dose-Response Analysis: Quantitative Points of Departure (PODs)

Commented [HT29]: calculation of the HEC through application of a DAF is considered to address the toxicokinetic but not the toxicodynamic component of the animal-tohuman extrapolation.

The fairly limited animal inhalation toxicity data identified by the literature search and PODs from the studies reviewed summarized in Table **Y**. All of the identified data are from animal studies and therefore need to be extrapolated to estimate the human inhalation exposure (EPA, 1994). Previously, the exposure duration adjustment was described. EPA has also developed guidance focused on improving the science underlying the animal-to-human uncertainty factor provides generalized procedures for deriving dosimetric adjustment factors (DAF) (EPA, 1994; 2002). Application of DAFs to the animal airborne exposure values yields estimates of the concentration that would result in the same concentration to humans, that is, the Human Equivalent Concentration (HEC). Application of a DAF in the calculation of a HEC is considered to address the toxicokinetic aspects of the animal-to-human UF (i.e., to estimate from animal exposure information the human exposure scenario that would result in the same dose to a given target tissue) (EPA, 2002). This procedure involves the use of species-specific physiologic and anatomic factors relevant to the form of pollutant (*e.g.*, particle or gas) and categorized with regard to elicitation of response . These factors are all employed in determining the appropriate DAF. For

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HECs, DAFs are applied to the "duration-adjusted" concentration to which the animals were exposed (e.g., to a weekly average). The generalized DAF procedures may also employ chemical-specific parameters, such as mass transport coefficients, when available.

The Regional Deposited Dose Ratio (RDDR) was used to derive DAFs for each of the surfactants with available animal toxicity studies. The RDDR is the ratio of the deposited dose in a respiratory tract region (r) for the laboratory animal species of interest (RDD_A) to that of humans (RDD_H) and was derived according to EPA's "*Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry*" (EPA, 1994). EPA's RDDR software allows calculation of calculate RDDRs in various regions of the respiratory tract for animals versus humans (*i.e.*, extra-thoracic, tracheobronchial, pulmonary, thoracic, total respiratory tract and extra-respiratory regions). The RDDR calculation is based on the characteristics of the aerosol tested in the inhalation study (Median Mass Aerodynamic Diameter or MMAD, Geometric Standard Deviation or GSD), animal species, animal mass, gender, etc. The RDDR selected as the DAF is informed by the effects (clinical signs, tissue effects, biochemical changes) observed in the animal toxicity study and the aerosol characteristics in the inhalation study. The summary of RDDR inputs (*e.g.*, MMAD and GSD) and results are provided in Table Y for each of the toxicity studies from which PODs could be identified.

For the nonionic surfactant, Oxynonal 9 (Triton-X 100), the effects observed (increased lung weights, alveolar/bronchiolar epithelial hyperplasia and lung inflammation) are consistent with lung effects in the LRT such that the pulmonary region RDDR (0.564) was used to calculate the HEC. For the anionic surfactant, oleoylsarcosine, the effects were seen in multiple regions of the respiratory tract, including squamous metaplasia and epithelium proliferation and submucous

acute inflammation at the base of the epiglottis and early stages of fibrosis in the alveoli walls. Therefore, total respiratory tract RDDR (1.504 for males and 0.970 for females) was used to calculate the HEC. In both 21- and 90-day inhalation studies with DDAC, effects observed (changes in BALF LDH, BALF total protein, BALF cell count (males only), increase in mucus in the respiratory epithelium, increase in hemorrhage, and increase in mucoid exudate, inflammatory cell infiltration and interstitial pneumonia) were indicative that the pulmonary RDDR (0.42 for 21-day exposure and 0.5 to 0.6 for 90-day exposure) is appropriate for calculating the HEC. In contrast, for the cationic surfactant, benzalkonium chloride histopathological cellular changes were observed in the nasal cavity and lungs, indicating the total respiratory tract RDDR should be used to calculate the HEC. The RDDRs applied and HECs derived from the animal study PODs are provided in Table Y.

TABLE Y HERE – SEE SEPARATE FILE

Benchmark Margin of Exposure Analysis

The analogues shown in Table X provide representative examples of the types of PODs that may be applied to new chemistries that meet the Surfactant Criteria. Though the initial starting point for deriving a benchmark MOE is based on a composite of the default values of 10 for each of the individual values for UF_H, UF_A, and UF_L, refinements may be warranted based on dosimetric adjustments to the applied concentrations used for establishing the experimental PODs. As shown in Table Y, the data-derived uncertainty factors, RDDRs were used as DAFs to account for animal-to-human toxicokinetic difference.

In the case of surface-active substances like chemical substances meeting the Surfactant Criteria, EPA has recently adopted a generalized approach that has historically been applied on a case-by-case basis for chemical substances, in recognition that surface-active effects that lead to irritation/corrosion do not require absorption, metabolism, distribution, or elimination (ADME) (EPA, 2019). In the context of this publication, irritation/corrosion include those effects in the respiratory tract that lead, for example, to inflammation, hyperplasia, and metaplasia. For chemical substances that act *via* a surface-active adverse outcome pathway (AOP), the default values for UF_H and UF_A are reduced to 3 (*i.e.*, $10^{0.5}$ or 3.162) to account for the uncertainty/variability for toxicodynamics, whereas the toxicokinetic component is reduced to 1 because ADME differences that would otherwise influence toxicokinetic differences are generally not relevant for surface-active substances. In order to apply these reductions, the following criteria must be established:

Commented [HT31]: Need Citation at end of this paragraph...assume it is the OPP Guidance??

1. A description of the AOP,
2. A discussion of why the AOP is unlikely or likely to differ between humans, in the case of UF_H, or between animals, in the case of UF_A, and
3. A discussion as to why the ADME of the chemical substance is unlikely to play a role in the observed toxicity.

When the above criteria are met, application of the appropriate dosimetric adjustment factor (*i.e.*, RDDR) should still be applied, given that deposition is the most appropriate dosimetric for assessing acute/subacute effects from surface-active agents. However, when dosimetric adjustments are applied, the reduction in the toxicokinetic component for UF_A are subsumed by the overall reduction, that is, no additional reductions should be incorporated.

Based on these information and criteria, the following composite values are appropriate to describe intra- and interspecies uncertainty/variability (*i.e.*, $UF_H \times UF_A$):

UF_H = 10 or 3: The default value of 10 should be applied when the available information does not support each of the above criteria. If the available information supports all of the above criteria, then a value of 3 may be applied.

UF_A = 10 or 3: The default value of 10 should be applied when the available information does not support the application of a dosimetric adjustment factor to quantifying a human equivalence concentration (HEC) or when the available information does not support each of the above criteria. If the available information allows derivation of an HEC and/or application of the above criteria, then a value of 3 may be applied.

UF_L = 10 or 1: If the POD from the experimental study is based on a LOAEC, then a default value of 10 should be applied, unless there is information to support that a reduced value is warranted. If the experimental data are amenable to benchmark dose modeling, a BMCL should be calculated and a value of 1 should be applied for this area of uncertainty.

Taken together, the above considerations and approaches support application of a benchmark MOE ranging from 10 to 1,000 and will depend on the analogue used and available data on the new chemical substance. In those instances where the data are too limited to determine when an analogue is appropriate for extrapolating the hazards to the new chemical substance,

experimental testing should be performed to aid with informing the quantitative assessment, as discussed under the Tiered-Testing Strategy.

Uncertainties and Limitations

The assessment framework outlined herein includes a number of uncertainties and limitations, include those associated with extrapolating the hazards identified from the analogues shown in shown in Table Y. Uncertainties associated with using animal studies to estimate human toxicity are recognized and methods developed to reduce them (OECD, 2014). Exposure duration adjustment procedures for inhalation exposures and application of DAFs to derive HECs, are well-established procedures for reducing uncertainties associated with the toxicokinetic aspects of animal-to-human extrapolation (EPA, 1994; EPA 2002). factors and derivation of benchmark MOEs (*i.e.*, type and magnitude of uncertainty factors). Likewise, EPA has recommended that BMD modeling be employed whenever possible to identify a POD and to reduce uncertainties associated with using a LOAEL from a toxicity study.

Commented [ST32]: OECD, 2014. [HYPERLINK "https://gcc01.safelinks.protection.outlook.com/?url=http%3A%2F%2Fwww.oecd.org%2Fofficialdocuments%2Fdisplaydocument%2F%3Fcote%3Denv%2Fjm%2Fmono(2014)4%26doclanguage%3Den&data=02%7C01%7CStedeford.Todd%40epa.gov%7C283d690eae994f6079e908d82dae913d%7C88b378b367484867acf976aacbeca6a7%7C0%7C0%7C637309575062395679&sdata=9%2BoEl9lB15HrNbOxTYXxIUBmTOrRyO5lCq4uT4rOiAM%3D&reserved=0" \t "_blank"], second edition Series on Testing and Assessment No. 194, 2014

[HYPERLINK "https://gcc01.safelinks.protection.outlook.com/?url=https%3A%2F%2Fwww.oecd.org%2Fenv%2Fehs%2Frisk-assessment%2Fgroupingofchemicalschemicalcategoriesandread-across.htm&data=02%7C01%7CStedeford.Todd%40epa.gov%7C283d690eae994f6079e908d82dae913d%7C88b378b367484867acf976aacbeca6a7%7C0%7C0%7C637309575062400652&sdata=RKUKYR%2FGjw%2FOunS0Tg9CIA2m4KqTzS%2BWoahkuxLHz6o%3D&reserved=0"]

Given the small number of chemical substances that meet the Surfactant Criteria that have concentration-response inhalation toxicity data, the applicability of these analogues to new chemical substances needs to be carefully considered, particularly given the influence of additional functional groups that may increase/decrease the toxicity of the new chemical substance compared to the comparator analogue. Risk assessors should first consider the surface tension and CMC criteria provided in Table X, and compare them to these measurements for the new chemical substance, if available, or the influence additional functional groups present or absent from the new chemical would have on these criteria (*e.g.*, would a particular functional group increase or

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decrease hydrophobicity or hydrophilicity and thereby increase or decrease CMC?). If such structural differences are judged not to significantly influence properties and toxicity, such that the new chemical substance is expected to have comparable or lower toxicity, read-across is an appropriate approach for characterizing hazards and risk. Of course, uncertainties regarding read-across should be acknowledged in the risk characterization.

For instances where the notifier of the new chemical substance and/or EPA is unable to conclude that one of the analogues in Table Y is comparable to or represents a worse-case analogue compared to the new chemical substance, then the Tiered-Testing Strategy provided herein should be employed to inform whether the new chemical substance has lower, comparable, or higher toxicity to the most representative analogue in the respective subcategory. Prior to conducting such testing, the scientific basis for selecting an analogue as the comparator compound to the new chemical substance should be understood and a rationale provided as to why the analogue is anticipated to have comparable or higher toxicity than the new chemical substance.

Commented [ST34]: William comment: "Surface tension and p-chem data may be able to rank the potency of the surfactants within a group."

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Use of New Approach Methods (NAMs) and *In Vitro* Testing Strategies to Avoid Excessive Animal Testing

The amended TSCA requires EPA to reduce reliance on animal testing using methods and strategies that "provide information of equivalent or better scientific quality and relevance for assessing risks of injury to health or the environment" (EPA, 2016). Additionally, in 2019, EPA wrote a directive to prioritize efforts to reduce animal testing by using NAMs (Wheeler, 2019). Multiple NAMs exist which can be used to assist in the hazard and risk assessment of new chemical substances that meet the Surfactant Criteria, including validated OECD methods for *in*

vitro irritation testing, as well as new *in vitro* methods to specifically assess respiratory toxicity. While several of the methods are described below, it is understood that this field is quickly advancing. Therefore, additional NAMs that are not described below may be discussed with EPA during a pre-notice consultation meeting.

Surfactants are proposed to cause a specific sequence of biological events in the pulmonary region if they are manufactured or used in a respirable form (*i.e.*, $\leq$ 10 µm). Therefore, an initial consideration of the potential for a surfactant to cause pulmonary toxicity is whether it is respirable. Several validated methods exist for making this determination (*e.g.*, cascade impactor, laser methods, OECD TG 110 and OPPTS 830.7520). As a practical matter, we propose using a cutoff of > 1% respirable particles/droplets by weight (wt%) for data obtained with these assays on the surfactant and/or a mixture containing the surfactant. This cutoff is consistent with EPA's "trace amounts" threshold for the nonreportable content for nanoscale materials (EPA, 2017).

If a surfactant is respirable, the next step with evaluating its potential to cause pulmonary toxicity would typically be *in vivo* inhalation assays; however, one approach for utilizing non vertebrate testing methods includes establishing a framework of events called an AOP. An AOP is an analytical construct that describes a sequential chain of causally linked (key) molecular or cellular events that lead to an adverse health effect that affects the organism and provides key information that may be used for informing quantitative risk assessment without the use of data obtained from vertebrate animals or, at a minimum, reducing the types of vertebrate animal data needed.

AOPs are the central element of a toxicological knowledge framework being built to support chemical risk assessment based on mechanistic reasoning (Leist et al, 2017). Representative key elements of AOPs are the molecular initiating events (MIEs), cellular level events (CLEs), organ or tissue level events (OLEs), and organism consequent events (OCEs). For surfactants, the crucial initial key event is proposed to be the interaction of the substance with lung-surfactant (MIE) and/or the molecular interaction of the substance itself with cell membranes (MIE), resulting in the disruption of lung cells due to loss of lung cell surfactant function (CLE) and/or the loss of membrane integrity (CLE). These initial events may lead to different OLEs (*e.g.*, alveolar collapse, loss of barrier function, blood extravasation, and impaired oxygenation of blood), which may finally lead to organism consequences (OCE) such as e.g. pneumonia, limited lung function by chronic obstruction (COPD), fibroses, etc.

Commented [KA36]: Arch Toxicol . 2017 Nov;91(11):3477-3505. doi: 10.1007/s00204-017-2045-3.

In vitro tests, such as by capillary surfactometer, may be useful in preliminary screening of chemicals to be tested, but do not by themselves constitute adequate tests for acute pulmonary effects of these chemicals. Therefore, if comparable concentrations are used in *in vitro* models, there will be a probability to get an overprediction in the results. This information should be taken into consideration within the design of additional *in vivo* tests.

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In vitro systems may help to investigate specific key events in the AOP and confirm that the substance may act like a typical surfactant (group assignment *via* similar AOP) and/or if other substance specific properties lead to a predominant type of key events within the AOP. Further, *in vitro* tests may also deliver information for avoiding *in vivo* testing (*e.g.*, corrosive substances cannot be tested due to animal welfare reasons) or providing helpful information on dose selection for *in vivo* testing, if needed. These assays can be used as part of a weight of scientific

evidence evaluation under Section 26(i) of TSCA, to determine whether animal testing is needed or if a point of departure (POD) can be determined for risk assessment purposes without the use of animals. These tests may also provide insight on the AOP.

Based on the AOP framework above, a number of different types of *in vitro* test methods, summarized in Table XX, may provide potentially useful information for informing the various elements of the surfactant AOP.

Table XX. *In Vitro* Test Methods That May Be Useful for Evaluating the AOP for Lung Effects of Surfactants.

Surfactant AOP	*Information on AOP*	*In Vitro* Assay	Test System
MIEs	MIE for interaction with pulmonary surfactant/loss of function	Specific *In Vitro* Respiratory Toxicity Assays	• *In vitro* lung surfactant inhibition as described by Sorli et al., (2017)
	MIE for interaction/penetration through cell membrane	*In Vitro/Ex Vivo* Irritation Assays	• OECD *In vitro/Ex Vivo* eye irritation tests for penetrance, *e.g.*: (OECD 492) Reconstructed human Cornea-like Epithelium (RhCE), (OECD 437) Bovine Corneal Opacity and Permeability Test, (OECD 438) Isolated Chicken Eye Test, *etc*.
CLEs	CLE for loss of membrane integrity/general cytotoxicity	*In Vitro/Ex Vivo* Cytotoxicity Assays	• OECD *In vitro/Ex Vivo* eye irritation tests for cytotoxicity, *e.g.*: (OECD 492) Reconstructed human Cornea-like Epithelium (RhCE), (OECD 437) Bovine Corneal Opacity and Permeability Test, (OECD 438) Isolated Chicken Eye Test, *etc*. • Cell membrane integrity test (LDH-lactate dehydrogenase cytotoxicity assay), MTT assay or lysosomal membrane integrity test. • BALB/c3T3/A549 lung cells neutral red uptake (NRU) cytotoxicity test, a test for basal cytotoxicity [HYPERLINK "https://ntp.niehs.nih.gov/iccvam/docs/acutetox_docs/brd_tmer/at-tmer-complete.pdf"]
OLEs	OLE for tissue level events	Human organotypic airway epithelial cultures	• ~~EpiAirway™~~-3-D constructs of human-derived cell cultures of differentiated airway epithelial cells (e.g., EpiAirway™ or MucilAir EpiAirway™, SmallAir™, etc. • ~~MucilAir EpiAirway™ 3-D constructs of human-derived cell cultures of differentiated airway epithelial cells~~
	OLE for tissue level events	Specific *Ex Vivo* Respiratory Toxicity Assays	• Precision-cut lung slice test *etc*. as described by Hess et al (2016)

[PAGE]

MIEs

The surfactant AOP is assumed to consist of two MIEs that may be informed by *in vitro* assays to determine whether a particular chemistry causes adverse effects on the pulmonary surfactant system (MIE #1), pulmonary cell membranes (MIE #2), or both. For MIE #1, Sorli et al., (2017) developed an *in vitro* lung surfactant inhibition assay that specifically measures whether the substance interferes with lung surfactant function. The assay was initially benchmarked for predicting the effect of waterproofing agents that were shown to be acutely toxic to mice. The authors noted that it may be overly conservative for some substances. Nevertheless, this assay investigated a basic principle (MIE #1) which may also be relevant for some types of surfactants. For MIE #2, the *in vitro* eye irritation assays represent appropriate screening approaches for determining the ability of surfactants to interact with cellular membrane and penetrate through the corneal layer of the eye. For example, Bader et al., (2013) showed that the BCOP assay was effective at identifying the potential for nonionic (*i.e.*, Triton X-100), anionic (*i.e.*, SDS), and cationic (*i.e.*, benzylalkonium chloride) substances to cause irritation to the eye; however, the authors also noted that the endpoints evaluated in this assay should be carefully assessed independently. For Triton X-100 and SDS, the permeability score was more predictive of eye irritation than the ocular opacity score, whereas for benzylalkonium chloride, the opacity score was more predictive of eye irritation than the permeability score. Therefore, a systematic investigation with surfactants using this approach may be helpful with elucidating MIE #2 of the AOP. In addition, information on the potential of a substance to cause *in vitro* skin irritation (e.g. OECD TG439) and/ or *in vitro* skin corrosion (OECD TG 431, when available, can provide orthogonal evidence of the potential for a substance to cause similar irritant or corrosive effects

in respiratory tract cells. Importantly, substances that are found to be corrosive cannot proceed to *in vivo* testing due to animal welfare concerns. If the substance is found to be a severe irritant, subsequent *in vivo* testing, if warranted, should be designed to avoid severe irritation effects in animals. For example, acidic or alkaline substances can be pH-adjusted to neutral values to prevent pH-mediated irritation to animals during testing. Corrosion effects mediated by pH extremes should be distinguished from necrosis effects *via* membrane disruption, for example DDAC causes tissue effects in inhalation studies despite having a neutral pH value of 6.8-6.9 ([HYPERLINK "https://www.sigmaaldrich.com/MSDS/MSDS/DisplayMSDSPage.do?country=US&language=en&productNumber=34466&brand=SIAL&PageToGoToURL=https%3A%2F%2Fwww.sigmaaldrich.com%2Fcatalog%2Fproduct%2Fsial%2F34466%3Flang%3Den"]).

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CLEs

Several *in vitro/ex vivo* assays are available that may aid with informing CLEs on general cytotoxicity in the surfactant AOP. For general cytotoxicity, the ocular irritation/corrosion studies cited in Table XX provide one set of options using cell types that are known to be sensitive to the effects of surfactants. Further, the NRU test has a validated protocol by ICCVAM using the BALB/c3T3/A549 lung cells, so there are test acceptance criteria, potential modifications for volatile substances, and stopping rules (for insoluble substances) (ICCVAM Test Method Evaluation Report, 2006). In each assay, surfactants with inhalation toxicity data such as Triton-X 100 and benzylalkonium chloride may be used as positive controls to

benchmark the results, thereby reliable results for estimating the potential for surfactants to cause irritation and cytotoxicity.

OLEs

Commented [ST40]: Note, the SmallAir system should be added to the above table, as possible OLE test systems

Based on the results of the testing on the CLEs, it may be necessary to perform more robust testing, given the limitations of these assays. For example, the discussed assays measure single cell types, whereas human and animal airway epithelia are composed of multiple cell types that each have specialized functions. Several human airway models have been developed that allow for the assessment of multiple endpoints in three-dimensional culture systems. Two commonly employed systems include EpiAirway™ and MucilAir™ developed by MatTek Life Sciences and Epithelix, respectively, and are discussed below.

Organotypic airway epithelial cultures, such as EpiAirway™ and MucilAir™, provide a more physiological *in vitro* model system compared to *in vitro* cell lines (EPA, 2018). Unlike single cell lines, these organotypic cultures take on a pseudostratified morphology, develop tight junctions, differentiate into multiple cell types, including: basal cells, ciliated cells, and goblet cells; generate mucus, exhibit ciliary beating, have xenobiotic metabolizing capacity, and maintain cultural homeostasis for months. Because of these characteristics, the human airway models are expected to better represent the response of *in vivo* tissue to surfactant exposure than cell line cultures of a single cell type. Depending upon the level in the respiratory system where the site of contact / exposure is predicted to occur, using for example MPPD modeling for determining deposition, different 3D cell culture systems are available that are composed of the different cell types that occur at different anatomical sites in the respiratory tract. For example,

Commented [KA41]: Issue Paper
Evaluation of a Proposed Approach to Refine Inhalation Risk Assessment for Point of Contact Toxicity:
A Case Study Using a New Approach Methodology (NAM)
EPA's Office of Chemical Safety and Pollution Prevention
August 30, 2018

MucilAir™ provides 3D co-culture models of cells from nasal, tracheal or bronchial sites, as well as a co-culture of cells from small airways (SmallAir™). EpiAirway™ is composed of normal human tracheal/bronchial epithelial cells as a co-culture system with normal human stromal fibroblasts, and EpiAlveolar™ is a 3D co-culture model of the air-blood barrier produced from primary human alveolar epithelial cells, pulmonary endothelial cells and fibroblasts.

Exposure to aerosols at the ALI using a Vitrocell® exposure system is a lower throughput approach to *in vitro* two-dimensional exposure systems; however, it provides a more comparable exposure to real-life exposure scenarios for inhaled aerosols. Using ALI exposure, dilution into medium and interaction with medium components does not occur as it would in a submerged culture system. There is interaction of the aerosol with a mucus or surfactant layer if organotypic cultures are used, as there would be *in vivo*, thus more physiologically relevant.

Exposures of these organotypic cultures at the ALI can be combined with a number of assays for assessing cell function and viability. Measurement of transepithelial electrical resistance (TEER), LDH-release, and viability assays such as MTT or ATP assays have all been reported for use with these cultures. These assays are multiplexable on the same cultures. TEER measures epithelial integrity, including functionality of intercellular tight junctions. LDH-release measures loss of plasma membrane integrity, which is indicative of cytotoxicity, and MTT and ATP assays measure cell viability. MatTek Life Sciences recommends the MTT assay for use with their EpiAirway™ cultures and recommends the surfactant Triton X-100 at 0.2% concentration as a

positive control for cytotoxicity. These assays can also be used to determine an HEC, which may be used for quantitative risk assessment.

While significant progress has been made toward achieving the objectives to use of high-throughput in vitro assays and computational models based on human biology to evaluate potential adverse effects of chemical exposures (NAS 2007, NAS 2017), the investigation of effects using *in vitro* models of higher levels of biological organization remains challenging. All other things being equal, for relevancy to humans and for animal welfare considerations, the 3D human airway cell culture systems discussed above would be the test systems to be aspired. However, depending on a number of factors, including the type of substance and specific decision context, use of different alternative assays may be considered. For example, the precision-cut lung slice (PCLS) test measures multiple endpoints, such as LDH for cytotoxicity and IL-1α for pro-inflammatory cytokine release in *ex vivo* cultures of rodent lung slices, to determine whether a chemical is likely to be toxic to the respiratory tract by inhalation exposure (Liu et al., 2019) .

Commented [RAB42]: NAS 2007 Toxicity Testing in the 21st Century [HYPERLINK "https://www.nap.edu/catalog/11970/toxicity-testing-in-the-21st-century-a-vision-and-a"]

NAS 2017 Using 21st Century Science to Improve Risk-Related Evaluations [HYPERLINK "https://www.nap.edu/catalog/24635/using-21st-century-science-to-improve-risk-related-evaluations"]

Commented [RAB43]: Liu et al. 2019 [HYPERLINK "https://respiratory-research.biomedcentral.com/articles/10.1186/s12931-019-1131-x"]

PCLS contain intact alveoli, rather than monolayers of one or two cells types (co-cultures). Crucially, in contrast to organoids, cell types are present in the same ratios and with the same cell–cell and cell–matrix interactions as *in vivo*. PCLS are often utilized in toxicological and anatomical studies regarding contractility in relation to asthma and other respiratory illnesses, such as emphysema (Sanderson et. al. 2011). Therefore, physiological responses, other than cytotoxicity, that may be evoked by the surfactant may be monitored. One further advantage of PCLS is that the PCLS assay can be performed on multiple species to determine susceptibility.

Commented [SM44]: Michael J. Sanderson, Ph.D. Exploring lung physiology in health and disease with lung slices
Pulm Pharmacol Ther. 2011 October ; 24(5): 452–465.

The PCLS test system has been pre-validated in multiple, independent laboratories, and the results showed good correlation when translated from *in vivo* LC_{50} values (Hess et al., 2016). While this assay has not yet been systematically used for surfactants, it may be considered for such substances once a solid database is established. While considered an alternative test, this assay still requires use of laboratory animals, albeit that, compared to *in vivo* inhalation tests, this assay reduces the number of animals that would be needed to conduct dose response studies. From a rat lung (1 g), about > 200 slices can be prepared. In general, for 1 concentration, 2 slices are used, resulting in 100 different concentrations or repeats that can be tested with one sacrificed rat. Additionally, PCLS cultures are stable for up to 4 weeks and allows for exposures via media or air with additional adaptations. The PCLS system can be considered to be an additional tool in the inhalation toxicity assay tool box. The rationale for selection of the PCLS assay, as with any inhalation toxicity assay, should be scientifically justified in advance of initiating testing.

Uncertainties/Limitations

The previous assays discussed under each of the respective surfactant AOP elements (*i.e.*, MIEs, CLEs, and OLEs) represent assays that may inform the potential inhalation toxicity from these substances; however, there are several uncertainties/limitations with these assays that warrant discussion. Though some of these are discussed elsewhere for each of the above testing systems, as well as others (Clippinger et al., 2018), it is important to consider that these assays were not systematically tested using surfactants and benchmarked against *in vivo* inhalation toxicity data on surfactants. Though we have recommended specific assays for evaluating the surfactant AOP,

a priori to using any or all of these tests is whether they can provide data that are comparable to *in vivo* tests and are suitable and fit for purpose in quantitative risk assessment.

In this regard, approaches to evaluate the scientific confidence of test methods for hazard assessment and risk assessment have, and continue to, evolve. A fit for purpose framework, employing specific criteria to establish relevancy, reliability, variability, sensitivity, domain of applicability, *etc*., for evaluating and documenting the scientific confidence of a new method for use for informing specific decision context has emerged from the regulatory science community to address the challenges posed for validation of NAMs that provide scientific rigor, but that are also flexible and adaptable (Parish et al., 2020; Patlewicz et al., 2015, EPA 2020).

Commented [RAB45]: https://www.sciencedirect.com/science/article/pii/S0273230020300180
[HYPERLINK "https://www.sciencedirect.com/science/article/pii/S0273230020300180"]
[HYPERLINK "https://www.sciencedirect.com/science/article/pii/S0273230015000392"]
[HYPERLINK "https://www.epa.gov/sites/production/files/2020-06/documents/epa_nam_work_plan.pdf"]

Once such fit for purpose scientific confidence evaluations are documented, there are several ways that these assays can be used to avoid excessive animal testing. First, testing can be performed on the surfactant AOP to evaluate the potency of new surfactants versus a comparator surfactant (*i.e.*, positive control) within the relevant subcategory that has repeated concentration inhalation toxicity data. Second, depositional data using models such as RDDR or MPPD for determining the depositional fraction of the new surfactant may be used for test concentration estimation and for estimating a potency ratio. Finally, *in vitro* to *in vivo* extrapolations (IVIVEs) may be used to determine a HEC for quantitative risk assessment.

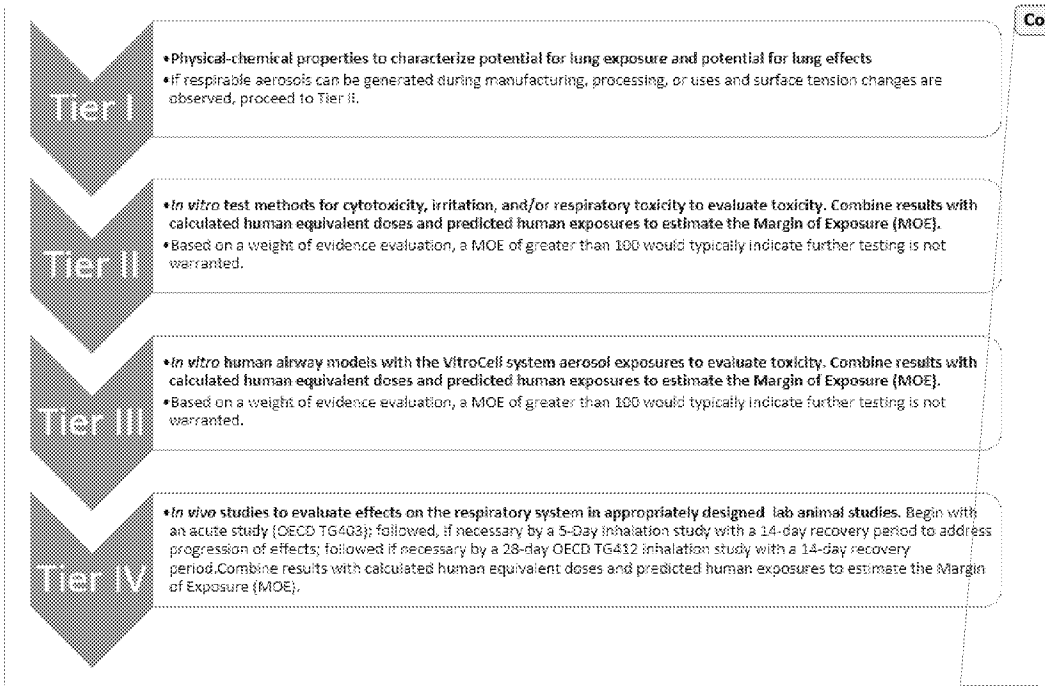
Commented [OS46]: Tala to include some additional text – read across, etc.

Reminder: Once text is set, refine text in the tiered testing figure

Tiered-testing Strategy

An approach to tiered testing is presented in Figure 1 and discussed in detail below. Drawing from the assays discussed above (and summarized in Table XX), this tiered testing and evaluation approach commences with the least complex, most efficient testing method, and then, at each subsequent tier, the complexity of the test system increases to more effectively emulate the biology and physiology of the *in vivo* respiratory tract system.

Draft Figure 1.



Commented [OS47]: Additional changes coming

Tier I—Physical-chemical properties

- Particle size distribution or aerosolized droplet size (*i.e.,* cascade impactor, laser methods) (OECD TG 110, Office of Prevention, Pesticides and Toxic Substances [OPPTS] 830.7520, OECD Guidance Document [GD] 39).

If respirable particles/droplets can be generated at greater than a mass fraction of 1 wt% during manufacturing, processing, or any of the uses for the new chemical substance, proceed to Tier II.

Commented [OS48]: Raphael: As per polymer overload, having a mg/m3 metric in addition to the 1% respirable would be helpful in certain situation e.g. very low particle/droplet emission during use so measuring 1% respirable is technically challenging or not feasible.

Commented [ST49R48]: I need to discuss this with Tala. The mg/m3 approach for this category is a bit more complicated than for the PLO category.

Tier II—*In vitro/Ex vivo* studies

The following *in vitro/ex vivo* test methods may provide potentially useful information ~~towards~~ with informing MIEs and CLEs. In order to determine the best approach for *in vitro*/*ex vivo* testing, a pre-notice consultation with EPA should be considered, given that none of the following studies are validated to determine lung toxicity induced by surfactants. In general, the testing approach should include a combination of assays, such as one on "Pulmonary surfactant interaction/loss of function", one on "Cell interaction/penetration", and one on "General cytotoxicity". The *in vitro*/*ex vivo* eye irritation studies may satisfy the latter two endpoints. If equivocal findings are obtained on the "Cell interaction/penetration" or "General cytotoxicity" assays, then the NRU cytotoxicity test should be performed. For each assay, the representative analogue to the new chemical substance for the respective subcategory of surfactants should be used as a positive control. Further, dosimetry models such as RDDR or MPPD should be used to simulate human exposures and to aid with identifying the appropriate test concentrations for the *in vitro*/*ex vivo* test systems,

considering for example the surface area of the culture system or *ex vivo* tissue, loss mechanisms, *etc*.

Pulmonary surfactant interaction/loss of function

- *In vitro* lung surfactant inhibition as described by Sorli et al., (2017)

Cell interaction/penetration

- OECD *In vitro* eye irritation tests, *e.g.*: (OECD 492) Reconstructed human Cornea-like Epithelium (RhCE), (OECD 437) Bovine Corneal Opacity and Permeability Test, (OECD 438) Isolated Chicken Eye Test, *etc*.

General cytotoxicity

- OECD *In vitro* eye irritation tests, *e.g.*: (OECD 492) Reconstructed human Cornea-like Epithelium (RhCE), (OECD 437) Bovine Corneal Opacity and Permeability Test, (OECD 438) Isolated Chicken Eye Test, *etc*.
- Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) recommended protocol for the BALB/c 3T3/A549 lung cells neutral red uptake (NRU) cytotoxicity test, a test for basal cytotoxicity (Appendix C1, [HYPERLINK "https://ntp.niehs.nih.gov/iccvam/docs/acutetox_docs/brd_tmer/at-tmer-complete.pdf"])

Each of the assays may be used to determine a starting point to calculate a modified POD_{HEC} using *in vitro* to *in vivo* extrapolation (IVIVE). The most sensitive of the endpoints identified

from the assays should be used to calculate a POD using BMD modeling, when possible, with the $BMCL_{1SD}$ metric. This metric is based on the benchmark response (BMR) of one standard deviation suggested for *in vitro* assays (a ~14.9% change from the control group value for the TEER assay), per the 2018 FIFRA Inhalation Scientific Advisory Panel meeting ([HYPERLINK "https://www.regulations.gov/docket?D=EPA-HQ-OPP-2018-0517"]). However, alternative metrics may be considered. For example, the pharmaceutical industry has utilized fixed adverse response thresholds that are appropriate for the specific biological assay (*i.e.*, EC_{15}, EC_{30}, *etc*; O'Brien 2006). Regardless of the metric used, a justification for its selection should be provided. ~~The *in vitro* POD can be converted to a deposited dose using the Multiple-Path Particle Dosimetry (MPPD) model for aerosols.~~ In those situations where data are not amenable to BMD modeling, due to assays that are not designed to provide concentration response data and/or lack sufficient granularity, the *in vitro* testing concentration level should be determined based on the expected HEC (taking into account the necessary MOE) to ensure that the *in vitro* data are generated in a concentration range relevant to the expected HEC. This alternative approach may be well suited when the expected human deposited dose is much lower than the typical/standard *in vitro* testing exposure dose.

Commented [ST50]: Note, I deleted this b/c of the statement above about using RDDR or MPPD for determining test concentrations.

When the data are amenable to calculating an HEC, the relevant routes of exposure should be considered, based on the conditions of use. ~~An margin of exposure~~MOE may then be determined by dividing the HEC by the estimated exposure and comparing to the benchmark MOE for the respective positive control.

Commented [RAB51]: I think this MOE sentence needs to be included to match up with the text in the tiered testing figure

It is not necessary to run both the cell interaction/penetration assay and the cytotoxicity assay; either one is sufficient for this tier. Based on the results of the above testing combinations, the following outcomes are possible, noting that a positive result in one of the ~~3 assay~~ endpoints identified in the assays, will drive the determination of "greater" or "comparable" toxicity to the positive control index chemical, whereas negative results ~~in all 3 assays~~for all of the evaluated endpoints will drive the determination of "lower" toxicity, as described below.

Greater Toxicity to the Index Chemical: ~~I~~If the new chemical substance exhibits greater toxicity compared to the positive control in one of the evaluated ~~assays~~endpoints, per the study method criteria, proceed to Tier III. For specific conditions of manufacturing, formulation, and use to consider engineering controls and/or appropriate PPE requirements for worker risks and/or reformulation of the new chemical substance at a lower wt% in products for consumer risks.

Comparable Toxicity to the Index Chemical: If the new chemical substance exhibits comparable toxicity to the positive control, per the study method criteria, in one of the evaluated assays, then stop at Tier II. It may be necessary, depending on the ~~margin of exposure~~MOE for specific conditions of manufacturing, formulation, and use to consider, for example, engineering controls and/or appropriate PPE requirements for worker risks and/or reformulation of the new chemical substance at a lower wt% in products for consumer risks.

Lower Toxicity to the Index Chemical If the new chemical substance exhibits lower toxicity or negative findings relative to the positive control, per the study method criteria, in all the evaluated assays, then determine if a modified POD_{HEC} can be calculated from the representative analogue in the respective subcategory of surfactants. If a modified POD_{HEC} can be calculated, then recalculate the MOE ~~reassess risks~~ using the modified POD_{HEC}. ~~using MOE as the risk metric~~ If risks are still identified with the modified POD_{HEC}, then stop at Tier II and consider engineering controls and/or appropriate PPE requirements for worker risks and/or reformulation of the new chemical substance at a lower wt% in products for consumer risks. If it is not possible to calculate a modified POD_{HEC}, then proceed to Tier III.

When deciding whether to proceed to additional testing, evaluation of the similarity of the substance to the index chemical is always needed. For example…. (Todd to fill in)

Tier III – Human Airway Models/PCLS Assay

- ~~Mat-Tek and/or Epithelix 3D human airway cells with VitroCell system aerosol exposures~~

~~*In vitro* to *in vivo* extrapolation to develop a $_{AEC}$ in Tier III is similar to the approach pursued in Tier II. The margin of exposure will be calculated by dividing the $_{AEC}$ by the exposure. While the exposure will be the same between Tier II and III, some uncertainty factors regarding the $_{AEC}$ can be avoided as the ALI-based exposure is more consistent with inhalation exposure in a human than the submerged culture exposures employed in Tier II (EPA, 2018). For inhaled surfactants the AOP is expected to be related to the physical chemical properties of these~~

Commented [KA52]: Issue Paper
Evaluation of a Proposed Approach to Refine Inhalation Risk Assessment for Point of Contact Toxicity:
A Case Study Using a New Approach Methodology (NAM)
EPA's Office of Chemical Safety and Pollution Prevention
August 30, 2018

Commented [OS53]: Stay consistent AOP not MoA – search throughout

Commented [ST54R53]: I deleted this because it seems redundant with the Category benchmark MOE discussion.

~~substances leading to impacts on lung surfactant or cell membranes. Because these effects are related to the concentration at the site of contact in the respiratory tract, this AOP does not require the typical ADME considerations used for selecting uncertainty factors for systemic toxicants. Instead, a default adjustment factor of unity for interspecies extrapolation for local effects via this AOP is considered to be scientifically justified (ECETOC 2014 http://www.ecetoc.org/wp-content/uploads/2014/08/ECETOC-TR-110-Guidance-on-assessment-factors-to-derive-a-DNEL.pdf).~~

Several testing options are available for evaluating OLEs in the surfactant AOP. The test system employed should focus on evaluating effects in the respiratory tract at the predicted sites of deposition (*e.g.*, TB and/or PU regions) using RDDR or MPPD modeling, as discussed previously. A justification for using a particular test system(s) versus another should be provided and may be discussed with EPA as part of a pre-notice consultation. Available test systems include, but are not limited to, the following:

- ~~EpiAirway™~~ 3-D constructs of human-derived cell cultures of differentiated airway epithelial cells for the relevant respiratory tract region where deposition will occur
  ~~MucilAir EpiAirway™ 3-D constructs of human-derived cell cultures of differentiated airway epithelial cells~~
- ~~Precision-cut lung slice assay~~test *etc.* ~~as described by Hess et al (2016)~~
-

Based on the results of the 3D-construct and/or PCLS testing, *in vitro* to *in vivo* extrapolation may be possible for developing a POD_{WEC} for use with characterizing potential risks using the MOE approach. Though the occupational/consumer exposure estimates may be the same

Commented [ST55]: I deleted this because it doesn't appear relevant to our situation. The ECETOC document specifies that the reduction to unity is for gases and vapors, not aerosols. See p. 29 of the cited document.

Commented [ST56]: Note, the SmallAir system should be included here

between Tiers II and III, the Tier III test results may offer the opportunity for refining the risk estimates. For example, the BMR used for calculating the POD_{BMC} may be refined because the ALI-based exposure is more consistent with inhalation exposure in a human than the submerged culture exposures employed in Tier II (EPA, 2018). Further, application of uncertainty factors for calculating the benchmark MOE may also be refined, if for example, human cultures are used, which may preclude the need for applying a UF_A.

Commented [KA57]: Issue Paper
Evaluation of a Proposed Approach to Refine Inhalation Risk Assessment for Point of Contact Toxicity:
A Case Study Using a New Approach Methodology (NAM)
EPA's Office of Chemical Safety and Pollution Prevention
August 30, 2018

If the Tier III test data are amenable for developing a POD_{BMC}, then the risk estimates should be reassessed. If no risks are identified under the conditions of use, then stop at Tier III. If risks are still identified under the conditions of use, then consider engineering controls and/or appropriate PPE requirements for worker risks and/or reformulation of the new chemical substance at a lower wt% in products for consumer risks.

If the Tier III test data are not amenable for developing a POD_{BMC}, then proceed to Tier IV. Again, as discussed above, when addition, when deciding whether to proceed to additional testing, evaluation of the similarity of the substance to the index chemical is needed. For example…. (Todd to fill in)

~~A margin of exposure of greater than 100 may mean that *in vivo* testing is not warranted. Additionally, if certain uses are controlled so that exposure is not a concern, these uses could be approved, and additional uses could require SNUR. If not, then meetings with toxicology experts~~

~~and EPA to discuss if further testing (*in vitro* or *in vivo*) is needed. Tier III and IV testing should only be done in consultation with EPA, and additional risk management options (e.g., engineering controls and personal protective equipment) should also be discussed. Even if additional *in vivo* testing is needed, these NAM assays can be used to determine a starting dose, potentially reducing animal testing.~~

Tier IV—*In vivo* studies

Strategic, tailored *in vivo* testing may be needed to inform the hazard and risk assessment of new chemical substances, particularly in those instances where 1) a new chemical substance has unique properties that preclude a determination that one of the subcategory analogues is appropriate for read across, as well as 2) ~~in~~ instances where the test data generated under Tiers II and III are not amenable for deriving POD_{MOS}. If *in vivo* testing is needed, a pre-notice consultation meeting with EPA should be considered prior to initiating any testing to discuss study design, exposure levels in relation to conditions of use, etc.

~~Note that a pronotification consultation with EPA should be considered prior to undertaking any Tier IV testing.~~

The potential for surfactants to cause adverse effects on the respiratory tract are based on acute toxicity concerns, that is, interfering with pulmonary surfactant and/or disrupting cellular membranes. Since these effects may be captured using appropriate exposure concentrations in short-term inhalation studies, the following *in vivo* tests are recommended:

- Step 1: OECD Acute TG 403 (modified)** featuring rats exposed for 4 hours and observed for 2 weeks using aerosol testing. ~~As described above, the HEC should be derived using default or chemical specific adjustment factors (CSAFs) and compared to potential actual human exposures to workers or consumers to determine a margin of safety or margin of exposure. Based on a weight of evidence evaluation in general, if the margin is > 100, further testing is not needed.~~

- Step 2: 5-Day inhalation study with a 14-day ~~recovery~~ observation period** to address progression of effects (use OECD TG 412, but conduct exposure duration for at least 5 days). ~~Proceed to step 3 if study reports substantial decrease in the POD over time relative to the acute study, or if an increase in lung burden is observed. The HEC should be derived using default or chemical specific adjustment factors (CSAFs) and compared to potential actual human exposures to workers or consumers to determine a margin of safety or margin of exposure. Based on a weight of evidence evaluation, in general, if the margin is > 100, further testing is not needed.~~

- ~~Step 3: OECD TG 412** 28-day inhalation study in rats with a 14-day recovery period.~~

Commented [RAB58]: Todd will update to add "stop" criteria for the 1-day acute study

Commented [ST59]: Recommend deleting, if there are concerns for effects in the respiratory tract consistent with the surfactant AOP, they will show up in the 5-day inhalation study.

**Modifications to all of the above studies should (if measureable) include pulmonary function testing, analysis of BALF, LDH release, blood oxygen (pO_2) content, ~~and satellite reversibility.~~ OECD TG 412 and OECD GD 39 should be consulted. Additionally, the sensory irritant potential can be measured using ASTM E 981 to determine reflex inhibition (Alarie et al., 2001).

Commented [KA60]: Alarie, Y., G.B. Nielsen, and M.M. Sch bioassays for evaluation of indoor air quality. *Quality Handbook*. Spengler, J.D., J.M J.F. McCarthy (eds.). New York: McGra pp 23.21–23.49.

The results of the *in vivo* testing may be used for reassessing and re-characterizing the previously identified risks under the conditions of use for the new chemical substance. Depending on the outcome of the risk assessment, EPA will apply risk management actions on those conditions of use that result in findings of unreasonable risk, whereas no restrictions would be applied on the conditions of use where the MOEs meet or exceed the benchmark MOE.

Commented [RAB61]: Todd to work on this

CONCLUSIONS

[To be added once text is finalized]

ASSOCIATED CONTENT

(Word Style "TE_Supporting_Information"). **Supporting Information**. A listing of the contents of each file supplied as Supporting Information should be included. For instructions on what should be included in the Supporting Information as well as how to prepare this material for publications, refer to the journal's Instructions for Authors.

The following files are available free of charge.

brief description (file type, i.e., PDF)

brief description (file type, i.e., PDF)

AUTHOR INFORMATION

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript. ‡These authors contributed equally.

Funding Sources

Any funds used to support the research of the manuscript should be placed here (per journal style).

Notes

Disclaimer: The views expressed in this article are those of the authors and do not necessarily represent the views or policies of their respective employers. Mention of trade names or commercial products does not constitute endorsement for use.

ACKNOWLEDGMENT

Generally, the last paragraph of the paper is the place to acknowledge people, organizations, and financing (you may state grant numbers and sponsors here).

Message

From: Richard E. Engler, Ph.D. [rengler@lawbc.com]
Sent: 6/19/2019 1:22:06 PM
To: Morris, Jeff [Morris.Jeff@epa.gov]
CC: Pierce, Alison [Pierce.Alison@epa.gov]; Scheifele, Hans [Scheifele.Hans@epa.gov]; lbergeson@lawbc.com; Heidi Brown Lewis [hlewis@lawbc.com]; Henry, Tala [Henry.Tala@epa.gov]; Schmit, Ryan [schmit.ryan@epa.gov]
Subject: RE: Webinar in July

Jeff:

Thank you for the update at the award ceremony that OCSPP will send a speaker. Has OCSPP decided if you or Alex will speak during this webinar?

Rich

RICHARD E. ENGLER, PH.D.
DIRECTOR OF CHEMISTRY
BERGESON & CAMPBELL PC
2200 Pennsylvania Avenue, NW, Suite 100W | Washington, D.C. 20037
T: 202-557-3808 | F: 202-557-3836 | lawbc.com

From: Morris, Jeff <Morris.Jeff@epa.gov>
Sent: Monday, June 10, 2019 11:15 AM
To: Richard E. Engler, Ph.D. <rengler@lawbc.com>
Cc: Pierce, Alison <Pierce.Alison@epa.gov>; Scheifele, Hans <Scheifele.Hans@epa.gov>; Lynn L. Bergeson <lbergeson@lawbc.com>; Heidi Brown Lewis <hlewis@lawbc.com>; Henry, Tala <Henry.Tala@epa.gov>; Schmit, Ryan <schmit.ryan@epa.gov>
Subject: RE: Webinar in July

Rich,

Thanks for the offer to participate. We will discuss within the office this week and get back with you soon.

All the best,

Jeff

From: Richard E. Engler, Ph.D. <rengler@lawbc.com>
Sent: Friday, June 07, 2019 2:48 PM
To: Morris, Jeff <Morris.Jeff@epa.gov>
Cc: Pierce, Alison <Pierce.Alison@epa.gov>; Scheifele, Hans <Scheifele.Hans@epa.gov>; lbergeson@lawbc.com; Heidi Brown Lewis <hlewis@lawbc.com>
Subject: Webinar in July

Jeff:

On July 25, Bergeson & Campbell, P.C. will be hosting a webinar to follow-up on topics discussed in the TSCA at 3 ELI workshop. The current working title of the webinar is **TSCA at 3: Overview With a Focus on Evolving New Chemical Policies and Practices**. The discussion will capture quickly key points from ELI conference and then pivot to Section 5 issues with a goal of identifying specific challenges and what stakeholders can do to avoid them.

B&C would be pleased if you could participate to discuss OPPT's views on these issues.

Please advise of your availability to join B&C for this webinar or if another day that week would be better for you.

Rich

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Message

From: Richard E. Engler, Ph.D. [rengler@lawbc.com]
Sent: 6/10/2019 3:15:09 PM
To: Morris, Jeff [Morris.Jeff@epa.gov]
CC: Pierce, Alison [Pierce.Alison@epa.gov]; Scheifele, Hans [Scheifele.Hans@epa.gov]; lbergeson@lawbc.com; Heidi Brown Lewis [hlewis@lawbc.com]; Henry, Tala [Henry.Tala@epa.gov]; Schmit, Ryan [schmit.ryan@epa.gov]
Subject: RE: Webinar in July

Sounds good!

See you at the ceremony this afternoon.

RICHARD E. ENGLER, PH.D.
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Message

From: Osman-Sypher, Sahar [Sahar_Osman-Sypher@americanchemistry.com]
Sent: 7/24/2020 9:28:54 PM
To: Stedeford, Todd [Stedeford.Todd@epa.gov]
CC: Henry, Tala [Henry.Tala@epa.gov]; Irwin, William [Irwin.William@epa.gov]; Salazar, Keith [Salazar.Keith@epa.gov]
Subject: RE: General Surfactants Manuscript Draft - July 23 Version 4
Attachments: draft manscript general surfactants - 23 July 2020.ver.4.docx

Todd – I have not received any further comments on the attached draft. Please note July 23, Version 4 is the latest version for surfactants. Folks will review over the weekend and be prepared with any thoughts Ex. 5 Deliberative Process (DP) Ex. 5 Deliberative Process (DP) on Monday's call.

Mike is still working on Ex. 5 Deliberative Process (DP) and I should get that later this evening and will forward.

Sahar

From: Osman-Sypher, Sahar
Sent: Friday, July 24, 2020 8:28 AM
To: 'Stedeford, Todd' <Stedeford.Todd@epa.gov>
Cc: Henry, Tala <Henry.Tala@epa.gov>; Irwin, William <Irwin.William@epa.gov>; Salazar, Keith <Salazar.Keith@epa.gov>
Subject: General Surfactants Manuscript Draft - July 23 Version 4

Todd – I received minor edits from Rick Becker to address the comments from ScitoVation. Ex. 5 Deliberative Process (DP) and and changed filename to July 23, Version 4. I will circulate this version to the team to review.

Mike/Wayne are working on Ex. 5 Deliberative Process (DP) and I should have an updated draft circulated later today.

Sahar

From: Stedeford, Todd [mailto:Stedeford.Todd@epa.gov]
Sent: Friday, July 24, 2020 6:43 AM
To: Osman-Sypher, Sahar <Sahar_Osman-Sypher@americanchemistry.com>
Cc: Henry, Tala <Henry.Tala@epa.gov>; Irwin, William <Irwin.William@epa.gov>; Salazar, Keith <Salazar.Keith@epa.gov>
Subject: RE: General Surfactants Manuscript Draft - July 23 Version 2 and Associated Tables/Figure

Here is a revised draft. Ex. 5 Deliberative Process (DP)

Ex. 5 Deliberative Process (DP)

From: Osman-Sypher, Sahar <Sahar_Osman-Sypher@americanchemistry.com>
Sent: Thursday, July 23, 2020 12:03 PM
To: Stedeford, Todd <Stedeford.Todd@epa.gov>
Cc: Henry, Tala <Henry.Tala@epa.gov>; Irwin, William <Irwin.William@epa.gov>; Salazar, Keith <Salazar.Keith@epa.gov>
Subject: General Surfactants Manuscript Draft - July 23 Version 2 and Associated Tables/Figure
Importance: High

Todd:

Attached is the latest version of the manuscript (July 23, Version 2) with discussions from the call incorporated. I've also added the updated tables and tiered testing figure.

Regards, Sahar

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Surfactants Category: The Application of New Approach Methodologies (NAMs) for Assessing Inhalation Risks under the Amended Toxic Substances Control Act

Commented [HT1]: Should intro have a bit more related to exposure? And how to fit in the irritation/corrosion properties of surfactants relative to inhalation?

Tala R. Henry[a,‡], *Keith Salazar*[b,‡], *Michael P. Hayes*[c], *Wayne Kennedy*[d], *Athena M. Keene*[d], *Annie Jarabek*[e], *Stefan Moors*[f], *Lela Jovanovich*[g], *Raphael Tremblay*[c], *Ann Tveit*[f], *Richard A. Becker*[h], *Sahar Osman-Sypher*[h], *Patrick D. McMullen*[i], *Scott D. Slattery*[i], *William Irwin*[b], *Marc Odin*[j], *Julie Melia*[j], *and Todd Stedeford*[a,*]

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KEYWORDS (Word Style "BG_Keywords"). If you are submitting your paper to a journal that requires keywords, provide significant keywords to aid the reader in literature retrieval.

ABSTRACT

[To be added after co-authors feedback] The abstract should briefly state the problem or purpose of the research, indicate the theoretical or experimental plan used, summarize the principal findings, and point out major conclusions. Abstract length is one paragraph.

INTRODUCTION

The Toxic Substances Control Act (TSCA) is the primary chemicals management law in the United States and was enacted to ensure the protection of health and the environment against unreasonable risks of injury from chemical substances. In 2016, the Frank R. Lautenberg Chemical Safety for the 21st Century Act (Pub. L. 114-182; hereinafter the "Lautenberg amendments") was signed into law, thereby amending TSCA. The Lautenberg amendments included substantial changes to EPA's

authorities and responsibilities under TSCA, including requirements on EPA to make determinations on new chemical substances for unreasonable risk, sufficiency of information with determining risk, and exposure-based risk determinations. The amended TSCA also included provisions mandating the reduction and replacement of vertebrate animals in testing, to the extent practicable and scientifically justified, in support of making a determination of unreasonable risk for new and existing chemical substances. TSCA section 4(h) also charges EPA with encouraging and facilitating:

> (1) the use of scientifically valid test methods and strategies that reduce or replace the use of vertebrate animals while providing information of equivalent or better scientific quality and relevance that will support regulatory decisions under TSCA;
> (2) the grouping of 2 or more chemical substances into scientifically appropriate categories in cases in which testing of a chemical substance would provide scientifically valid and useful information on other chemical substances in the category; and
> (3) the formation of industry consortia to jointly conduct testing to avoid unnecessary duplication of tests, provided that such consortia make all information from such testing available to the Administrator.

The present investigation advances each of these TSCA mandates for chemical substances characterized as surfactants.

A surfactant is a substance that reduces the surface tension of a liquid in which it is dissolved. They are surface-active, amphiphilic compounds that self-assemble to form micelles or aggregates above a critical concentration, referred to as the critical micelle concentration (CMC). These substances are commonly used in occupational settings, in consumer products (*e.g.*,

household cleaning products, personal care products, *etc*.), and in biological research and development (R&D) as detergents, wetting agents, emulsifiers, foaming agents, and dispersants. Their use in such applications provide pathways of exposure by which potential toxicity of these compounds may occur to human or environmental receptors. Specifically, the inherent properties of surfactants may induce toxicity if exposures occur such that they can interfere with biological surfactants or tissues. For example, sodium dodecyl sulfate, a strong anionic surfactant, is used in R&D applications at concentrations up to 10% to disrupt cell membranes and to denature proteins, whereas octylphenoxypolyethoxyethanol, a mild nonionic surfactant, is used in R&D applications up to 1% to disrupt cell membranes, while preserving proteins for isolation (Burden, 2012).

Hazard concerns for surfactants were historically focused on their observed environmental effects and potential toxicity to aquatic organisms (Cowan-Ellsberry, 2014). For example, the U.S. Environmental Protection Agency (EPA) established chemical categories for cationic (quaternary ammonium) and anionic surfactants based on environmental toxicity concerns (EPA, 2010). Surfactants may also be a potential hazard concern to humans, depending on the use and route of exposure, because they can disrupt the normal architecture of the lipid bilayer and reduce the surface tension, thereby solubilizing cell membranes. For example, mucous membranes are particularly sensitive to the surface-active effects of surfactants, which have been shown to cause irritancy and injury to the eye, based on their ability to "readily penetrate the sandwiched aqueous and lipid barriers of the cornea" (Fox and Boyes, 2008).

Depending on the conditions of use, inhalation exposures to workers and/or consumers may be possible that warrant consideration in quantitative risk assessments. As noted, surfactants may cause adverse effects on mucous membranes, including the respiratory tract, and have been shown to interfere with the natural pulmonary surfactants, resulting in reduced oxygen content of arterial blood (*i.e.*, impaired gas exchange in the lung), increases in pulmonary extravascular water volume and wet-to-dry weight ratio of the lungs, grossly visible pulmonary edema, and atelectasis (Nieman and Bredenberg, 1985; Wang et al., 1993; Modell et al., 1969). However, the chemical space for surfactants that may present inhalation hazards has not been previously defined, and the potential for inhalation toxicity ranges by orders of magnitude, such as Octoxynol 9, a nonionic surfactant (Triton-X 100; CASRN 9002-93-1; 14-day lowest-observed-adverse-effect concentration [LOAEC] of 5.3 mg/m^3) (EPA, 2016; ECHA, 2020), versus didecyldimethyl ammonium chloride, a cationic surfactant and biocide (DDAC, CASRN 7173-51-5; 4-week lowest-observed-adverse-effect concentration [LOAEC] of 0.08 mg/m^3 for portal-of-entry effects) (MDEQ, 2003; CIR, 2003; ECHA, 2020).

The purpose of the present investigation was to: (1) perform a systematic review of the literature with the aim of defining the chemical space for surfactants; (2) identify appropriate toxicological analogues, when available, for identifying potential inhalation hazards and when data allow, identifying quantitative point(s) of departure for use in an inhalation risk assessment; (3) describe scientifically sound new approach methodologies (NAMs) to reduce or replace animal testing, where possible; and (4) establish a tiered-testing strategy, that utilizes NAMs, as appropriate, for new chemistries in the surfactant space.

MATERIALS AND METHODS

Systematic Literature Review

Commented [OS2]: Todd to summarize and move the details to an appendix

Objective

The objective of the literature search, screening, and retrieval process was to obtain studies that evaluated the toxicity of surfactants in the lower respiratory tract (LRT or thoracic region; *i.e.*, tracheobronchial and pulmonary regions) in exposed humans, investigated LRT outcomes in laboratory animals, or informed an adverse outcome pathway or mode of action for these agents at a cellular level (*i.e.*, *in vitro* studies). Because a list of surfactants with Chemical Abstracts Service Registry Numbers (CASRNs) was not known *a priori*, the initial PubMed search strategy was broad, with the intention of capturing potentially relevant information on any surfactant compound. Additional search strategies were employed to obtain studies not identified by keyword searching using Medical Subject Headings (MeSH or mh) and text words (tw) in PubMed.

PubMed Search

Computerized literature searches were initially conducted in PubMed in November 2016 to obtain studies related to the toxicity of surfactants in the LRT of humans and experimental animals. The search query string is presented in Table 1.

Table 1. PubMed search strategy for lung effects of surfactants.

Database Search Date	**Query String**[a]
PubMed 11/15/2016	("surface-active agents"[mh] AND lung[mh]) AND ((detergents[mh] OR aerosols[mh] OR "pulmonary surfactants"[mh]) OR (lung diseases[mh] OR cell respiration[mh] OR surface tension[mh]))

[a] Note, an Updated Literature Search was performed in April 2018, which excluded an expanded list of MeSH, query, and text words. Further details are provided in the Supplemental Information file titled "[redacted]".

Screening methods for this search included manual screening of titles/abstracts and screening of full text articles using the PECO criteria shown in Table 2.

Table 2. PECO criteria for screening of literature search results for lung effects of surfactants.

PECO element	**Evidence**[a]
Population	Humans, laboratory animals (rats, mice, hamsters, guinea pigs, dogs, non-human primates, or other inbred mammals) and mammalian cell lines
Exposure	*In vivo* (all routes), *ex vivo* (isolated perfused lung), and *in vitro*
Comparison	Any comparison (across dose, duration, or route) or no comparison (*e.g.*, case reports without controls)
Outcomes	Any examination of: • Pulmonary effects *in vivo* or *ex vivo* studies • Cytotoxicity or alternative methods in *in vitro* studies

[a] The PECO criteria were refined and more specific in the Updated Literature Search performed in April 2018. For more details, see the Supplemental Information file titled "[redacted]".

Additional Search Strategies (Gray Literature, Tree Searching, and Literature Search)

A search of the gray literature[1] was performed in September 2018 to obtain additional information pertaining to lung effects of surfactants. Resources searched for pertinent gray literature are listed in Table 3. The chemicals and compound groups identified from the initial literature search and used for gray literature searching are listed in Table 4. Screening methods for this search included manual screening of titles/abstracts and full text reports using the PECO criteria shown above in Table 2.

Table 3. List of resources to search for gray literature.

ATSDR [HYPERLINK "http://www.atsdr.cdc.gov/toxprofiles/index.asp"]
Chemtrack [HYPERLINK "http://www.chemtrack.org/White/CMR.pdf"]
CIR [HYPERLINK "http://www.cir-safety.org/ingredients"]
ECETOC publications [HYPERLINK "http://www.ecetoc.org/publications"]
ECHA [HYPERLINK "http://echa.europa.eu/web/guest/information-on-chemicals/registered-substances"]
EFSA (European Food Safety Authority) [HYPERLINK "http://www.efsa.europa.eu/"]
EPA – ChemView (incl. TSCATS data) [HYPERLINK "https://chemview.epa.gov/chemview"]
EPA – HPV Hazard Characterization Documents [HYPERLINK "http://iaspub.epa.gov/oppthpv/hpv_hc_characterization.get_report?doctype=2"]

[1] Gray literature, as used herein, has the same meaning as defined by EPA (2018) and "refers to sources of scientific information that are not formally published and distributed in peer-reviewed journal articles. These references are still valuable and consulted in the TSCA risk evaluation process. Examples of gray literature are theses and dissertations, technical reports, guideline studies, conference proceedings, publicly-available industry reports, unpublished industry data, trade association resources, and government reports."

Table 3. List of resources to search for gray literature.

EPA – HPV Risk-Based Prioritization Documents (RBPs) [HYPERLINK "http://iaspub.epa.gov/oppthpv/hpv_hc_characterization.get_report?doctype=1"]
EPA – HPVIS via ChemID - [HYPERLINK "https://chem.nlm.nih.gov/chemidplus/chemidlite.jsp"]
EPA – TSCATS 1 (available via Toxline)
EPA – pesticides - [HYPERLINK "https://iaspub.epa.gov/apex/pesticides/f?p=CHEMICALSEARCH:1"] Archive [HYPERLINK "https://archive.epa.gov/pesticides/reregistration/web/html/status.html"]
FDA [HYPERLINK "https://www.fda.gov/default.htm"]
HERA [HYPERLINK "http://www.heraproject.com/RiskAssessment.cfm"]
HSDB [HYPERLINK "http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB"]
INCHEM (CICADS, EHC, HSG, IARC, IPCS, JECFA, SIDS) [HYPERLINK "http://www.inchem.org/"]
JECDB (Japan Existing Chemical Data Base) [HYPERLINK "http://dra4.nihs.go.jp/mhlw_data/jsp/SearchPageENG.jsp"]
NICNAS http://www.nicnas.gov.au/
NITE [HYPERLINK "http://www.safe.nite.go.jp/jcheck/search.action?request_locale=en"]
NTP [HYPERLINK "https://ntpsearch.niehs.nih.gov/home"]
OECD [HYPERLINK "http://www.echemportal.org/echemportal/page.action?pageID=9"]
OECD/SIDS [HYPERLINK "http://webnet.oecd.org/hpv/ui/SponsoredChemicals.aspx"]

Table 3. List of resources to search for gray literature.

ATSDR = Agency for Toxic Substances and Disease Registry; CICADS = Concise International Chemical Assessment Document; CIR = Cosmetic Ingredient Review; ECETOC = European Centre for Ecotoxicology and Toxicology of Chemicals; ECHA = European Chemicals Agency; EFSA = European Food Safety Authority; EHC = Environmental Health Criteria; EPA = Environmental Protection Agency; FDA = Food and Drug Administration; HERA = Human and Environmental Risk Assessment; HPV = High Production Volume; HPVIS = High Production Volume Information System; HSDB = Hazardous Substances Data Bank; HSG = Health and Safety Guideline; IARC = International Agency for Research on Cancer; INCHEM = Internationally Peer Reviewed Chemical Safety Information; IPCS = International Programme on Chemical Safety; JECDB = Japan Existing Chemical Data Base; JEFCA = Joint Expert Committee on Food Additives; NICNAS = National Industrial Chemicals Notification and Assessment Scheme; NITE = National Institute of Technology and Evaluation; NTP =National Toxicology Program; OECD = Organisation for Economic Cooperation and Development; SIDS = Screening Information Data Set; TSCATS = Toxic Substances Control Act Test Submissions

Table 4. Surfactants, constituent names, and CASRNs to use for searching gray literature.

Chemical Group or Constituent Name	**CASRN**
Alkoxysilane resins	Not applicable; chemical group term
Defomaire	No data
Alevaire OR tyloxapol	25301-02-4
Triton X-100 OR polyethylene glycol p-isooctylphenyl ether	9002-93-1
Dioctyl sodium sulfosuccinate (DOSS) or butanedioic acid, 2-sulfo-, 1,4-bis(2-ethylhexyl) ester, sodium salt (1:1)	577-11-7
Polyoxyethylene-10-oleyl ether (C18:1E10)	9004-98-2
Polyoxyethylene-10-dodecyl ether (C12E10)	6540-99-4
N,N-dimethyl-dodecylamine-N-oxide (C12AO)	1643-20-5

The reference lists of the primary studies and review articles identified by the PubMed search were manually screened to identify additional pertinent literature for lung effects of surfactants (*i.e.*, tree searching). An Updated Literature Search was performed in April 2018. The details of

this search are provided in the Supplemental Information file titled "___". This literature search was used to identify additional studies or data related to LRT effects of surfactants that became available after the original search was conducted.

Risk Assessment Approaches under TSCA

Risk Assessment Paradigm

The current methods and approaches of risk assessment, both across EPA and as articulated in TSCA, have been built upon decades of expert development, scientific peer review, refinement, and scientific knowledge. Generally, EPA conducts risk assessments following the four-step process articulated by the National Research Council in 1983 (NRC, 1983) and reaffirmed as an appropriate approach several times since (NRC, 1994; NRC, 2009). This process includes hazard identification, dose-response analysis, exposure assessment, and risk characterization. Hazard assessment (also called effects assessment in some EPA guidance documents) identifies the types of adverse health or environmental effects or hazards that can be caused by exposure to the chemical substance in question and characterizes the quality and weight of scientific evidence supporting this identification. In the dose-response assessment, the relationship between the exposure or dose of a chemical and the occurrence of health or environmental effects or outcomes is assessed. The exposure assessment characterizes the extent of human or environmental exposures, including the magnitude, frequency, and duration of the exposure, to the extent necessary and practicable within the context of the assessment. Finally, the risk characterization integrates the hazard, dose-response, and exposure assessment to describe the nature, and when possible, the magnitude of risks to human health and the environment.

The approaches employed for these components, including, for example, the level of detail and complexity of quantitative aspects may vary across different risk assessments and typically align with specific legislative and regulatory frameworks. For example, legislative and regulatory frameworks for hazard evaluation of pesticide active ingredients, anti-microbial substances, inerts, *etc*. are described in regulations for pesticides, which include multiple and specific requirements for toxicity data. Under TSCA and its implementing regulations (see EPA's Review Process for New Chemicals, 2020), companies are required to submit a Premanufacture Notice (PMN) along with all available data on: chemical identity, production volume, byproducts, use, environmental release, disposal practices, and human exposure. These submissions are required to include all existing health and environmental data in the possession or control of the submitter, parent company, or affiliates, and a description of any existing data known to or reasonably ascertainable by the submitter. However, TSCA has never included requirements for toxicity testing or generation of hazard data for new chemical substances prior to submission for review by EPA.

Commented [RAB3]: https://www.epa.gov/reviewing-new-chemicals-under-toxic-substances-control-act-tsca/epas-review-process-new-chemicals

Hazard Assessment

Given the lack of toxicity testing requirements under TSCA, EPA only occasionally receives empirical hazard data for new chemical substances. EPA recently conducted an analysis of toxicity tests submitted to EPA for new chemical substances under TSCA and found that ___% of PMN submissions included any type of toxicity testing and most were for aquatic toxicity. TSCA provides EPA with the authority to require generation and submission of additional data when the information included with the PMN, coupled with that available to EPA risk assessors from prediction modeling, read-across, internal archives, *etc*. is insufficient to permit a reasoned

Commented [HT4]: Website name; DIFFERENT THAN NAME OF DOCUMENT, which is really looong.

evaluation of the health and environmental effects of a new chemical substance. However, prior to making a request for testing using vertebrate animals, EPA must take into consideration reasonably available existing information, including toxicity information; computational toxicology and bioinformatics; and high-throughput screening methods and the prediction models of those methods (TSCA Section 4(h)(A)(i)-(iii)).

Given the historical lack of hazard data and the new requirements to consider reasonably available existing information, EPA has, for decades, relied on a number of approaches that do not rely on *de novo* toxicity testing, including computational toxicology (*e.g.*, predictive models and expert systems), analogue read-across (wherein available toxicity data for a chemical of similar structure and activity is used to assess the new chemical substance lacking data), and chemical categories (a group of chemicals whose properties are likely to be similar or follow a regular pattern as a result of mechanism, mode of toxic action or structural similarity) (van Leeuwan et al., 2009).

Commented [HT5]: van Leeuwen, K., Schultz, T.W., Henry, T., Diderich, B., Veith, G. 2008. Using chemical categories to fill data gaps in hazard assessment. *SAR and QSAR in Environ Res*, 20:207-220.

1.Dellarco, V., Henry, T., Sayre, P., Seed, J., Bradbury, S. 2010. Meeting the common needs of a more effective and efficient testing and assessment paradigm for chemical risk management. *J Toxicol Environ Health*, 13:347-360.

Dose-Response Analysis

For assessing hazards to human health, EPA relies most heavily on read-across methods using an analogue or a category of analogues to identify hazards and conduct dose-response analysis to identify a point of departure (POD). While EPA has a number of existing "TSCA New Chemicals Program (NCP) Chemical Categories" (EPA, 2010), including for anionic, nonionic, and cationic surfactants, the existing surfactant categories were developed and defined based only on environmental toxicity considerations. Toxicity tests for analogues are used to identify a point of departure (POD) (*i.e.*, a dose or concentration that marks the beginning of a low-dose

Commented [HT6]: EPA, 2020. TSCA New Chemicals Program (NCP) Chemical Categories. Office of Pollution Prevention and Toxics, Washington, DC.

[HYPERLINK "https://www.epa.gov/sites/production/files/2014-10/documents/ncp_chemical_categories_august_2010_version_0.pdf"]

Anionic Surfactants pg. 34//Eco only

Cationic (quaternary ammonium) Surfactants pg. 51//Eco Only

Nonionic Surfactants pg. 94//Eco only

extrapolation) for assessing risks to the new chemical substance. This point can be the lower bound on dose for an estimated incidence or a change in response level from a dose-response model (*i.e.*, benchmark concentration or dose [BM(C)D], NOAE(C)L, LOAE(C)L, or human equivalent concentration or dose [HE(C)D]) for an observed incidence or change in level of response) (EPA, 2017).

Once suitable analogues are identified, the strengths, limitations, and uncertainties associated with using the analogue as predictive of hazards of the new chemical substance are considered to derive a benchmark margin of exposure (MOE). The benchmark MOE is the result of multiplying all relevant uncertainty factors (UFs) to account for: (1) the variation in susceptibility among the members of the human population (*i.e.*, inter- individual or intraspecies variability); (2) the extrapolation from animal data to humans (*i.e.*, interspecies extrapolation); (3) the extrapolation from data in a study with less- than- lifetime exposure (*i.e.*, extrapolating from sub-chronic to chronic exposure); (4) the extrapolation from a LOAEL rather than from a NOAEL; and (5) the potential derivation of an under-protective value as a result of an incomplete characterization of the chemical's toxicity (EPA, 2002, 2011). EPA prefers using existing information to set the magnitude of the UF value (EPA, 2014). However, data-derived UFs (known as data derived extrapolation factors – DDEFs or chemical specific adjustment factors – CSAFs) are not often possible, especially for new chemical substance, thereby requiring the use of default UFs.

Exposure Assessment

Commented [HT7]: RfD/RfC Guidance has a really nice figure showing the duration and DAF adjustments… include??

In assessing new chemical substances, EPA typically generates the human exposure estimates for workers using modeling approaches including the Chemical Screening Tool for Exposures and Environmental Releases (ChemSTEER). ChemSTEER exposure estimates are generated as daily

acute potential dose rates (PDRs) in mg/kg-bw/day or lifetime average daily doses (LADDs) in mg/kg-bw/day. Given that new chemical substances will not have occupational exposure monitoring data, except for possible monitoring data on analogues, the PDR is typically used as an initial conservative exposure estimate when calculating the MOE.

Due to the surface-activity of surfactants at the point of exposure, the PDR is the appropriate dose-metric. For chemical substances used in a liquid, mist, or aerosol form, the general default PDR value is 1.875 mg/kg-bw/day (*i.e.*, 15 mg/m^3; 1.875 mg/kg-bw/day × 80 kg-bw ÷ 10 m^3/day) (EPA, 2013 [ChemSTEER manual]). A summary of the default values used for calculating PDRs for new chemical substances in mist or aerosol form is provided in Table 6.

Table 6. Default values used for calculating the PDR.

Description	Equation	Description	Equation[a]	Defaults	Units
PDR (mg/kg-bw/day)	I/BW	Inhalation PDR (I)	Cm × b × h, where Cm is the mass concentration of chemical in air, b is the volumetric inhalation rate ($0 < b \leq 7.9$), and h is the exposure duration ($0 \leq h \leq 24$)	Cm = 15 mg/m^3 b = 1.25 m^3/hr h = 8 hours/day	mg/day
		Body weight (BW)	BW ($0 \leq BW$)	80 kg	Kg

[a] Cm may also be adjusted for the mass concentration of the chemical with a PEL in air (Based on OSHA PEL – TWA; default = 15 mg/m^3), the weight fraction of chemical in particulate(Ys) ($0 < Ys \leq 1$), the weight fraction of chemical or metal with a PEL in particulate (Ypel) ($0 < Ypel \leq 1$) using the following equation: Cm = KCk × Ys/Ypel

Occupational exposures are most often reported as 8-hr TWAs for exposures during workdays (5 days/week) and therefore, discontinuous exposures of animal studies are adjusted to derive HECs relevant to the occupationally exposed human population. The optimal approach is to use a physiologically-based pharmacokinetic model; however, the data required to conduct such modelling rarely exist for new chemical substances. Therefore, occupational exposures are adjusted using particle deposition models with human exertion (work) ventilation rates and exposure durations appropriate to the particular occupational setting and chemical use scenario. A duration adjustment is applied to the POD to account for the exposure conditions under evaluation (*e.g.*, workers = 8 hours/day, 5 days/week) versus the exposure conditions employed in the experimental study (*e.g.*, 6 hours/day, 5 days/week).

Commented [HT8]: (U.S. EPA, 1994).

Risk Characterization

Risk characterization is an integral component of the risk assessment process for both ecological and health risks, *i.e.*, it is the final, integrative step of risk assessment. As defined in EPA's Risk Characterization Policy, the risk characterization integrates information from the preceding components of the risk assessment and synthesizes an overall conclusion about risk that is complete, informative, and useful for decision makers. In essence, a risk characterization conveys the risk assessor's judgment as to the nature and existence of (or lack of) human health or ecological risks (EPA, 2000). As noted in EPA's Risk Characterization Handbook "Risk characterization at EPA assumes different levels of complexity depending on the nature of the risk assessment being characterized. The level of information contained in each risk

characterization varies according to the type of assessment for which the characterization is written and the audience for which the characterization is intended."

Risk characterization is performed by combining the exposure and dose-response assessments. Under TSCA section 5, EPA must determine whether a chemical substance presents an unreasonable risk of injury to health or the environment under the conditions of use. EPA generally uses an MOE approach to characterize risks of new chemical substances as a starting point to estimate non-cancer risks for acute and chronic exposures. The MOE is the HEC derived from a POD for a specific health endpoint (from hazard assessment) divided by the exposure concentration for the specific scenario of concern (from exposure assessment). To determine whether the resulting MOE results in an adequate margin between human exposure estimates and the HEC derived from a POD, the MOE value is compared with a pre-determined benchmark MOE. When using MOEs as risk estimates for non-cancer health effects, the benchmark MOEs are used to interpret the risk estimates. Human health risks are interpreted when the MOE is less than the benchmark MOE. On the other hand, negligible concerns would be expected if the MOE exceeds the benchmark MOE. Typically, larger MOEs (if greater than the benchmark MOE) result in a lower likelihood that a non- cancer adverse effect will occur. MOEs allow for providing a non-cancer risk profile by presenting a range of estimates for different non-cancer health effects for different exposure scenarios and are a widely recognized point estimate method for evaluating a range of potential non-cancer health risks from exposure to a chemical.

In summary, to conduct a risk evaluation for new chemical substances, as required under TSCA section 5, EPA conducts a hazard assessment, using empirical data when available, but most

often using analogues, to identify a POD(s) and to develop a benchmark MOE that reflects specific uncertainties associated with data available for use in the evaluation. This hazard assessment is combined with the exposure assessment, to calculate an MOE, which is compared to the benchmark MOE to determine whether risks are identified. The risk characterization is used to inform the "unreasonable risk" determination.

RESULTS AND DISCUSSION

Literature Search and Screening Results

The results of the literature search and screening effort are presented graphically in Scheme 1. The PubMed search identified 43 potentially relevant studies for full text review. The PubMed search results were supplemented by a search of gray literature resources, which identified six references for full text review. The Updated Literature Search identified nine additional studies for full text review.

The full text review of 60 references yielded X potentially relevant studies with data on lung effects of surfactants (*i.e.*, references that were cited in this white paper). Studies that were excluded following full text review included X papers on compounds that were not used as surfactants. Studies were also excluded if they did not evaluate lung effects (n = X; no evaluation of respiratory function and/or pathological examination of the lungs).

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Scheme 1. Literature search and screening flow diagram for surfactants

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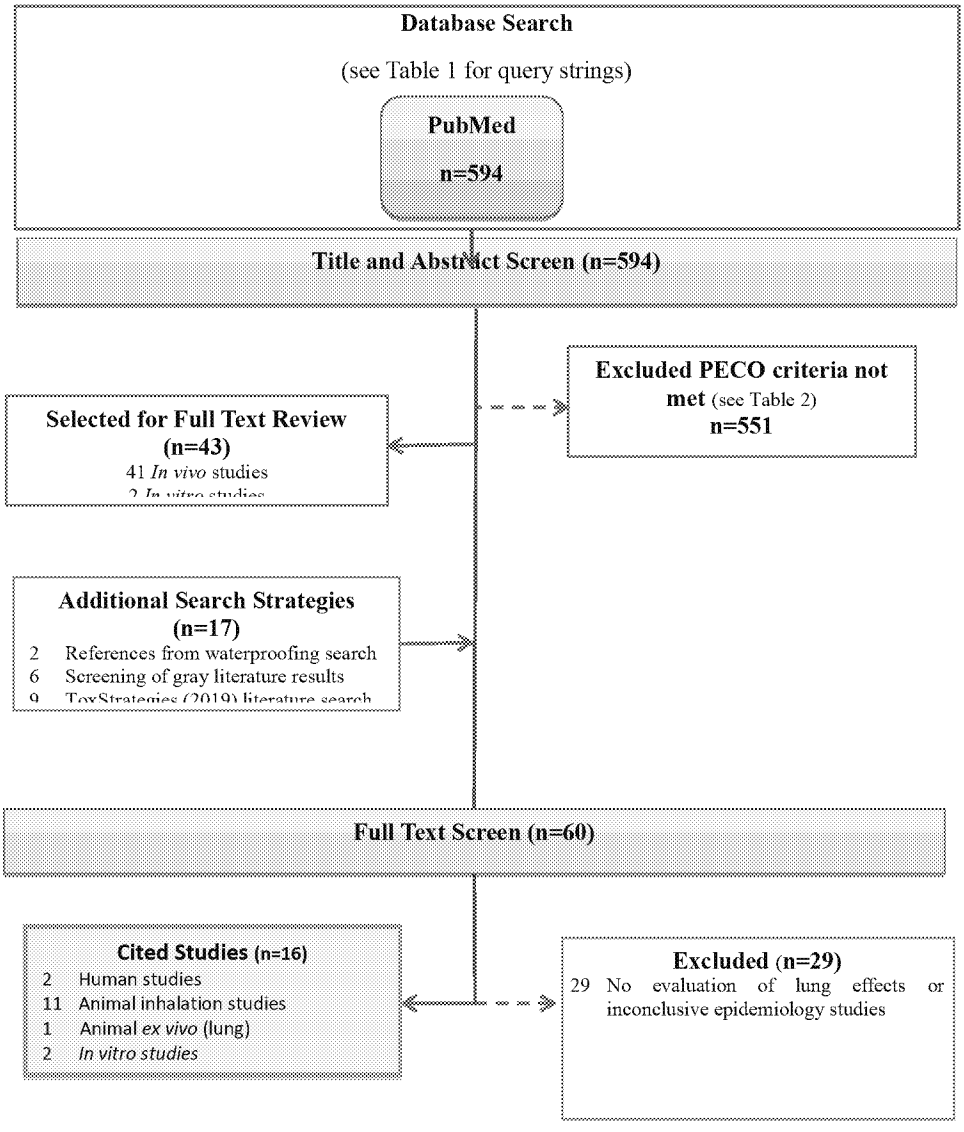

[PAGE]

Category Boundaries

Surfactants are comprised of three general subcategories including nonionic, anionic, and cationic substances. Within these subcategories, the following defined structural and functional criteria (hereinafter referred to as the "Surfactant Criteria") are used to distinguish chemical substances, which include polymers and UVCB substances,[2] intended for use as surfactants from other amphiphilic compounds (*e.g.*, ethanol) (EC, 2009, 2011; HTS, 2017):

1. A substance which has surface-active properties, and which consists of one or more hydrophilic and one or more hydrophobic groups;
2. The substance must be capable of reducing the surface tension between air and water to 45 milliNewtons/meter (mN/m) or below at a test condition of 0.5 wt% in water and a temperature of 20°C (*Cf.* Pure water has a surface tension of 72.8 mN/m at 20°C); and
3. The substance self-associates in water to form micellar or vesicular aggregates at a concentration of 0.5 wt% or below.

The Surfactant Categories were subcategorized for those chemical substances that initially meet the Surfactant Criteria and possess ionic or nonionic properties, as discussed below. Note, though not listed in the following subcategories, amphoteric chemical substances that meet the Surfactant Criteria would also be included within these subcategories (*i.e.*, cationic or anionic surfactants), depending on their pH. Lung lining fluids are near neutral pH, with various measurements ranging

[2] Chemical Substances of Unknown or Variable Composition, Complex Reaction Products and Biological Materials (UVCB Substance)

from 6.6 to 7.1 (Ng et al., 2004; Choudhary et al., Nielson et al., 1981). The pKa for each component of an amphoteric surfactant should be considered within this pH range and the assessment should be conducted on the predominant ~~or both~~ components. ~~The non-ionized fraction for acids/bases should be calculated as follows.~~

~~$\text{Acids Fraction}_{\text{Non-ionized}} = 1 / (1 + 10^{(pH - pKa)})$~~

~~$\text{Bases Fraction}_{\text{Non-ionized}} = 1 / (1 + 10^{(pKa + pH)})$~~

~~Where the pH represents the physiological pH in the lung (i.e., 6.6 to 7.1), and the pKa represents the value for the respective component (e.g., carboxylic acid or amine). A group has equal amounts of charged and neutral quantities at the pH value equal to the pKa value. At a pH value that is one unit below the pKa value, carboxyl groups are 10% negatively charged. At a pH value that is one unit above the pKa value, carboxyl groups are 90% negatively charged. At pH values below the pKa value, amine groups are positively charged. At a pH value that is one unit below the pKa value, amine groups are 90% positively charged. At a pH value that is one unit above the pKa value, amine groups are 10% positively charged. At physiological pH values, quaternary ammonium, phosphonium or sulfonium groups are positively charged while sulfonate and phosphonate groups are negatively charged.~~

Commented [KA11]: Should this sentence be deleted?

Commented [OS12]: Todd will update to simplify, show equation,
Aromatic amines is an aniline, ph7

Nonionic surfactants were identified as any neutral chemical substance that meets the Surfactant Criteria. Common nonionic surfactants include alkylphenol chemical substances with one or more than one ethoxylate (EO) unit as well as linear and branched alcohol chemical substances with one

or more EO units. Octoxyphenol with 9 EO units (CASRN 9002-93-1; a.k.a., octoxynol 9 or Triton-X 100), a common nonionic octylphenol EO surfactant and Polysorbate 80 or Tween 80 (CASRN 9005-65-6, another nonionic alkyphenol ethoxylate with increased alkyl chain length and number of EO units, are shown in Table X. The surface tensions of octoxynol 9, Polysorbate 20 and Polysorbate 80 have been reported as 30-31 mN/m at a concentration of 0.1% in water (33 mN/m, 1% actives at 25 °C) and 37.96 mN/m (0.5% at XX °C), respectively as shown in Table X (DOW, 2009, 2020; **Kothekar, et al., 2017**).

Commented [ST13]: Temp?

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Anionic surfactants were identified as any chemical substance with a net negative charge that meets the Surfactant Criteria (*e.g.*, alkyl sulfonates, alkylbenzene sulfonates, alkylether sulfates, alkyl silicic acids, alkyl phosphates, alkyl carboxylic acids, or combinations of these anionic groups). The structure of the common anionic surfactant SDS is shown in Table X. The surface tension of SDS is reported to be 39.5 mN/m at 25° C in water (Table X).

Commented [ST17]: Not in Mike's Table

Commented [ST18]: "The [HYPERLINK "https://en.wikipedia.org/wiki/Critical_micelle_concentration" \o "Critical micelle concentration"] (CMC) in pure water at 25 °C is 8.2 mM,[HYPERLINK "https://en.wikipedia.org/wiki/Sodium_dodecyl_sulfate" \l "cite_note-CMC-1"] and the [HYPERLINK "https://en.wikipedia.org/wiki/Aggregation_number" \o "Aggregation number"] at this concentration is usually considered to be about 62.[HYPERLINK "https://en.wikipedia.org/wiki/Sodium_dodecyl_sulfate" \l "cite_note-3"] The [HYPERLINK "https://en.wikipedia.org/wiki/Micelle" \o "Micelle"] ionization fraction (α) is around 0.3 (or 30%).[HYPERLINK "https://en.wikipedia.org/wiki/Sodium_dodecyl_sulfate" \l "cite_note-Barney_L-4"]"

[HYPERLINK "http://hera.ugr.es/doi/15008447.pdf"]
this paper shows ST to be a lot higher

Cationic surfactants were identified as any chemical substance with a net positive charge that meets the Surfactant Criteria (*e.g.*, alkylammonium chlorides and benzalkonium chlorides). The structure of the common cationic surfactant DDAC, as shown in Table X, is a representative member of this subcategory, although as noted previously, it also possesses biocidal properties. The surface tension of DDAC is reported to be 27.0 mN/m at 0.1% in water (Table X).

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[INSERT TABLE X]

Hazard Identification

There is concern for dysfunction of natural surfactant in the lung from inhalation of surfactants. Additionally, there is evidence that some surfactants or similar structures may also interfere with the cell membrane (Jelinek et al., 1998, Parsi et al., 2015). The capacity of exogenous surfactants to interfere with pulmonary surfactant and impair pulmonary function has been demonstrated in human volunteers and in laboratory animals. The pulmonary response to surfactant aerosol is in proportion to the exposure concentration and duration, but available data are inadequate to identify effect levels, which in any case are likely to vary not only with the specific chemical surfactant, but also with the exposure method (*e.g.*, aerosol droplet size).

Commented [ST22]: Add the following, based on Updated Literature Search?
Evander et al. 1988
Rao & Das 1994
Ekelund et al. 2004

Note, exposure conditions need to be presented in the studies, e.g., 6 hrs/day, 5 days/week. Also, units should be consistently presented, e.g., mg/L versus mg/m3

Commented [OS23]: Parsi et al Phlebology. 2015 Jun;30(5):306-15. doi: 10.1177/0268355514534648.

In vitro toxicity of surfactants in U937 cells: cell membrane integrity and mitochondrial function
A Jelinek H P Klöcking Exp Toxicol Pathol. 1998 Sep;50(4-6):472-6.

Nonionic Surfactants

Several studies were found for the nonionic siliconized superinone respiratory detergent, formaldehyde, polymer with oxirane and 4-1,1,3,3-tetramethylbutylphenol (CASRN 25301-02-4; also known as Defomarie, Alevaire, Tyloxapol). Healthy human volunteers showed significantly decreased pulmonary compliance following acute inhalation of Defomaire beyond that produced by the distilled water control (Obenour et al., 1963). Increased minimum surface tension due to detergent was demonstrated, and shown to be dose-dependent, using pulmonary surfactant extracted from dogs and mixed *in vitro* with the nonionic surfactant tyloxapol (Alevaire) (Modell et al., 1969). *In vivo* exposure of dogs to Alevaire in this study (8 h aerosol exposure; vehicle and concentration not reported) produced little effect (only 1/10 dogs exposed to Alevaire showed

Commented [OS24]: Patrick McMullen Comment; Defomaire, Tyloxapol, Alevaire, and Superinone all refer to the same substance, correct? Recommend that after the first sentence it should be referred to using the same "name" each time.

increased minimum surface tension), which the authors concluded support the dose-dependence of the effect and indicate that small amounts of detergent can be present in the lungs without detectably altering surfactant function (Modell et al., 1969).

Other pulmonary effects in dogs and/or sheep exposed to nonionic surfactant, tyloxapol, included reduced oxygen content of arterial blood (*i.e.*, impaired gas exchange in the lung), increases in pulmonary extravascular water volume and wet-to-dry weight ratio of the lungs, and grossly visible pulmonary edema and atelectasis (*i.e.*, collapsed alveoli) (Nieman and Bredenberg, 1985; Wang et al., 1993; Modell et al., 1969). In the study by Modell et al., (1969), no gross pathology differences were seen in detergent-exposed vs. control lungs of dogs, although some portions of both control and exposed lungs were heavy and discolored reddish-purple, which may have been caused by fluid accumulation from the liquid aerosol exposures and/or the use of hypotonic saline in the study (0.45% NaCl). Normal appearances were observed in the remaining areas of the lungs.

In rodent models, irritation and inflammatory effects on the respiratory tract has been observed with varying degrees of severity. Acute inhalation exposure to Polysorbate 20 via nose-only administration for 4 hours in Wistar Han rats to a concentration of 5.1 mg/l (5,100 mg/m^3) did not observed in mortalities, clinical signs, or abnormalities in the gross pathology[3]. Using MPPD modeling, the total lung deposition mass was calculated to be 6.6E+4 µg. A respiratory irritation study was conducted on a mixture containing Nonidet in male Webster mice using the ASTM Method E981 where animals were exposed for 3 hours to concentrations of 12, 22, 51, 118, and

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[3] [HYPERLINK "https://echa.europa.eu/hr/registration-dossier/-/registered-dossier/13525/7/3/3"]

134 mg/m^3 (Alarie and Stock, 1992, unpublished). Signs of respiratory irritation was observed in animals at the three highest concentrations as indicated by increased respiratory frequency without an increase in pulmonary edema or lung weight. An acute inhalation exposure study in Syrian hamsters to 3.0 mg/l of Triton X-100 to varying exposure durations reported that lung deposition of Triton X-100 corresponded to mortality with an LD50 of 1300-2100 µg (Damon et al., 1982). The authors concluded that the deaths in these animals were likely the result of severe laryngeal edema and ulcerative laryngitis while the lower airways and lungs in these animals were relatively free of serious pathologies. The authors hypothesized that that these observed effects were due to large tracheobronchial deposition following the aerosol exposure and the mucociliary clearance of the deposited chemical resulted in a large concentration of the chemical on the laryngeal mucosa. Finally, in the only repeated dose inhalation exposure identified for nonionic surfactants, a 2-week repeated dose inhalation study was conducted on Triton X-100 in male and female Sprague-Dawley rats to 5.3 mg/m^3 (MMAD 1.8 µm, GSD 1.8µm) for 6 hours/day, 5 days/week (Bio/dynamics, Inc. 1992[4].) Slight to minimal subacute inflammation of the alveolar walls and hyperplasia of the alveolar/bronchiolar epithelium was reported, in addition to an increase in slight discoloration of the lungs, increased lung weight, and mucoid nasal discharge.

Commented [SK25]: It is unclear to me if the other tested concentration should be included since it is a 70% mixture.

In vitro studies of surfactant effects on cell membranes have provided evidence of possible MOAs. Warisnoicharoen et al., (2003) evaluated the cytotoxicity of the nonionic surfactants polyoxyethylene-10-oleyl ether ($C_{18:1}E_{10}$), polyoxyethylene-10-dodecyl ether ($C_{12}E_{10}$), and N,N-

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[4] Bio/dynamics, Inc. 1992. A two week inhalation toxicity study of C-437 and C-1754 (ethoxylated para-tertiary-octyl phenol) in the rat with cover letter dated 5/24/96 (sanitized). NTIS Report No. OTS0573048.

dimethyl-dodecylamine-N-oxide ($C_{12}AO$; CASRN 1643-20-5) to cultured human bronchial epithelium cells (16-HBE14o-) *in vitro*, using the MTT cell viability assay. All of the surfactants tested were cytotoxic at concentrations near or below their critical aggregation (micellular) concentrations (as determined by surface tension measurements), suggesting that surfactant toxicity was due to the disruption caused by the partitioning of monomeric surfactant into the cell membrane.

Lindenberg et al (2019) evaluated the cytotoxic activity of the of three nonionic polymeric surfactants, which are commonly used in formulations of nebulized pharmaceuticals to prevent protein agglomeration, Polysorbate 20 (Tween 20), Polysorbate 80 (Tween (80) and Poloxamer 188in a BEAS-2B human bronchial epithelial cell model by using an innovative air-liquid interface (ALI) method of exposure compared to classical liquid/liquid (L/L) model. The study measured the release of Lactate Dehydrogenase (LDH) which is an intercellular enzyme present in large amounts in the cytoplasm. Loss of membrane integrity will cause the release of LDH into the extracellular medium. Cytotoxicity of Polysorbate 20 was observed at concentrations of 1-2% (v/v) when using the more biologically relevant ALI method by measuring Lactate Dehydrogenase (LDH) activity, however, a significant increase in LDH was only observed at 4% for Polysorbate 80 and not significantly increased at concentrations of up to 10% for Poloxamer 188. These results suggest that Polysorbate 20 and to the lesser extent Polysorbate 80 induce damage to the cell membrane integrity while the linear Poloxamer 188 did not demonstrate any in vitro cytotoxicity.

Altogether, the available in vitro and in vivo data indicate a wide discrepancy in respiratory toxicity among nonionic surfactants. The small dataset presented in this section preclude establishing

correlations between respiratory effects and chemical properties such as surface tension or CMC. Others have examined the relationship between chemical properties of nonionic surfactants and eye irritation and concluded that hydrophilic-lipophilic balance, pH, alkyl chain length, or poly [oxyethylene] chain lengths failed to predict eye irritation potential across the nonionic subcategory (Heinze et al., 1999). However, significant correlations of eye irritation and the maximum reduction in surface tension were observed at the CMC or higher surfactant concentration when conducted under nonequilibrium conditions. Whether this chemical property similarly predicts potency of nonionic surfactants to induce respiratory effects requires additional data and analysis outside of the scope of this summary.

Anionic Surfactants

Two acute inhalation toxicity studies were identified for several anionic surfactants which demonstrated high toxicity via the inhalation route. Oleoyl sarcosine was evaluated in a 4-hour nose only inhalation study in male and female Sprague-Dawley rats using concentrations of 0.3, 0.6, 2.2, and 3.7 mg/L. An LC_{50} of 1.37 mg/L was identified with edema of the lung at 0.6 mg/L and audible gasping at 0.3 mg/L. For Sodium Lauroyl Sarcosinate (CASRN 137-16-6), 5 male Wistar rats were exposed to a 4-hour nose-only inhalation concentration of 0.05, 0.5, 1, and 5 mg/L and 5 female rats were exposed to 1.1 or 5.5 mg/L. All 10 animals exposed to 5 mg/L died within 1-2 h of dosing, and 4/5 of the animals exposed to 0.5 mg/L and the 10 animals exposed to 1 mg/ml died within 1-2 days after dosing. Animals in the 0.05 mg/l had no clinical signs or mortality at the conclusion of the study. At necropsy, red foci were noted on the lungs in animals of groups receiving concentrations of ≥ 0.5mg/L. The LC_{50} was reported to be 0.05-0.5 mg/L.

Commented [OS26]: Mike/Wayne have indicated that this does not meet the boundary criteria. It is quite insoluble, etc. More information to follow.

Commented [OS27R26]: William will address this in the table re: oleoyl sarcosine and sodium salt version.

[PAGE]

Repeated-dose inhalation studies were identified for oleoyl sarcosine (CASRN 110-25-8), and dioctyl sodium sulfosuccinate (CASRN 577-11-7). Oleoyl sarcosine was evaluated in a 28-day nose-only inhalation study (OECD Guideline 412) in male and female Fischer rats (5/group/sex) using concentrations of 0, 0.006, 0.02, or 0.06 mg/L in 10% ethanol[5]. The mass median aerodynamic diameter (MMAD) of the aerosol particles were 1.11- 1.22 μm and the geometric standard deviation (GSD) was 1.68-2.57. Changes in the mean corpuscular volume (MCV), white blood cells (WBC), and lymphocytes in male animals of the high dose groups were observed. In female animals of the mid-dose group, reticulocyte counts were significantly reduced. Reflex bradypnea was noted in the animals of the mid and high doses which is associated with severely irritating substances. All test concentrations caused effects at several sites of the respiratory tract with indications for local irritation, such as squamous metaplasia and epithelium proliferation and submucous acute inflammation at the base of the epiglottis. In the lungs and bronchi, the most prominent finding was a focal early stage of fibrosis, but details were not provided at the dose level for this effect. Lung weights were increased at the highest dose. The NOEL was <0.006 mg/L (6 mg/m^3) air in males and females; the basis for the effect level was local irritation.

Commented [ST28]: Todd will add these footnotes (i.e., 3 + 4) to EndNote file

Dioctyl Sodium Sulfosuccinate was evaluated in a 13-week inhalation study in male and female Sprague-Dawley rats (12/group/sex), to an aerosol of a product containing of 4.2 mg/m^3, for 4 hours a day, 5 days a week[6]. There were no statistically significant differences in dosed and control

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[5] [HYPERLINK "https://echa.europa.eu/hr/registration-dossier/-/registered-dossier/21429/7/6/3"]

[6] Cosmetic, Toiletry, and Fragrance Association (CTFA). 1991. Acute oral, ocular, primary dermal irritation, 21-day dermal irritation, photocontact allergenicity,

groups, for the mean body weight gain, survival, appearance and behavior, urinalysis values, and microscopic lesions. Significant differences were noted in the blood such as elevated erythrocytic values in male rats at 7 weeks and depressed mean corpuscular hemoglobin concentration values in male rats at 13 weeks. At 7 weeks, the lungs of animals necropsied were stained with Oil Red O and examined; scattered foci of neutrophils and an increase in alveolar macrophages were reported in a single dosed male rat. A LOAEC of 4.2 mg/m^3 was identified based on blood effects in male rats.

Mechanistic studies examining the pulmonary effects of anionic surfactants have been studied in dogs and/or sheep exposed, dioctyl sulfosuccinate sodium salt. (DOSS; CASRN 577-11-7). Increased minimum surface tension of lung extract or bronchioalveolar lavage fluid (BALF) was observed in dogs and sheep following *in vivo* aerosol exposure to the anionic detergent dioctyl sodium sulfosuccinate (DOSS) in 1:1 mixture of ethanol and saline for 30 – 60 minutes, at a concentration that was selected to ensure a moderate degree of edema (estimated dose of 15 mg detergent/kg body weight) (Nieman and Bredenberg, 1985; Wang et al., 1993). Light microscopic examination of the lungs 4 hours after exposure to DOSS aerosol observed no grossly destructive effects on alveolar cells or lung architecture in exposed dogs. However, a decrease in pulmonary compliance was observed that the authors hypothesized was due to an increase in surface tension in the alveoli in the presence of detergent.

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6 RIPTs, 13-week subchronic dermal, 13-week subchronic inhalation, four 4-day mini-cumulative irritation. Submission of unpublished data by CTFA, 200 pp.

Pulmonary clearance studies using radiolabeled aerosol tracers have evaluated whether detergent effects on the surfactant layer lead to increased alveolar permeability. For example, inhalation exposure to DOSS enhanced the pulmonary clearance of radiolabeled diethylenetriamine pentaacetic acid (DTPA), a relatively small hydrophilic molecule, reflecting increased alveolar permeability after detergent exposure (Nieman et al., 1990; Nilsson and Wollmer, 1992, 1993; Evander et al., 1994; Tasker et al., 1996; Nilsson et al., 1997). In most studies, this effect on alveolar permeability was seen in the absence of effects on blood gas levels or pulmonary compliance that occur with higher exposure, indicating that the increase in alveolar permeability is a sensitive effect of detergent aerosol. The effect was demonstrated to be concentration-related in one study in which multiple dilutions of the liquid detergent were nebulized (Evander et al., 1994). Some studies also evaluated the clearance of a radiolabeled aerosol of albumin, a much larger molecule, which was enhanced by DOSS as well, but to a lesser degree than DTPA (Nilsson and Wollmer, 1992; John et al., 1997). Wang et al., (1993) observed an increase in protein flux from plasma to alveolar space after DOSS inhalation in sheep, which the authors attributed to disruption of the alveolar lining and increased microvascular permeability. The increased alveolar permeability observed in these studies has been hypothesized to result from increased alveolar surface tension, which could cause increased permeability either by opening previously closed pores (through which solutes pass) in the membrane or by stretching already open pores (Nieman et al., 1990; Wang et al., 1993). However, as previously mentioned, surfactants can disrupt cell membranes; thus, this mechanism may be an alternate explanation (Burden, 2012).

Cationic Surfactants

Acute Studies

Acute inhalation toxicity studies were identified for DDAC, Dioctadecyldimethylammonium chloride (DODMAC), and BAC. For DDAC, rats (5/sex/dose, unspecified strain) were exposed via inhalation to 0.05, 0.09, 0.13, 0.25, 1.36 mg/L, or 4.54 mg/L for 2 hours observed for 14 days. An LC_{50} of 0.07 mg/L was identified based on unspecified abnormalities identified in several organs including the lungs (EPA OPP RED). For DODMAC, Albino rats (10 males, strain not specified) were exposed to the test substance (1:29 distilled water) via inhalation at 180 mg/L for one hour and observed for 14 days (OECD SIDS, 1996). There were no mortalities. Treatment-related clinical signs included preening, excessive masticatory (chewing) movements, excessive salivation stains, lacrimation, serosanguineous stains around the nose and labored respiration. All animals appeared normal one day after dosing. The LD_{50} (1h) was > 180 mg/L. For BAC, female Wistar rats (5/group) were exposed via nose-only inhalation to 37.6 and 53 mg/m^3 for 4 hours and observed for 14 days or exposed to 30.6 mg/m^3 for 6 hours and BALF was measured 18 hours post-exposure (Swiercz et al., 2008). The identified LC_{50} was approximately 53 mg/m^3 and BALF analysis reported increased inflammatory markers such as TNF-a, IL-6 and an increase in indicators of lung damage such as LDH, total protein, and increased lung weight.

Repeated-Dose Studies

DDAC - didecyldimethyl ammonium chloride

Three repeated dose inhalation studies of three different exposure durations were identified for the cationic surfactant DDAC: 14-day, 20 to 21-day, and 90-day.

In the 14-day study, male Sprague-Dawley rats were exposed via whole-body inhalation exposures to DDAC aerosols of 0.15 mg/m^3, 0.6 mg/m^3, and 3.6 mg/m^3 (Lim et al., 2014). The

mass median aerodynamic diameter (MMAD) of the aerosols was 1.86 μm and the geometric standard deviation (GSD) was 2.75 μm. Mild effects were noted in the bronchoalveolar cell differentiation counts, cell damage parameters in the BAL fluids, in addition to inflammatory cell infiltration, and interstitial pneumonia of the medium and high groups. The NOAEC was determined to be 0.15 mg/m^3.

In the intermediate exposure study, male and female Sprague-Dawley rats (5 rats/sex/group) were exposed via dynamic nose-only inhalation for a total of 20 or 21 days to concentrations of 0, 0.08, 0.5, and 1.5 mg/m^3 (Weinberg, 2011). The MMAD was 1.4-1.9 μm and the GSD was 1.83-1.86 μm. Lung weights were increased in females in the mid- and high-concentration groups and in males in the high concentration group. The bronchoalveolar lavage fluid (BALF) analysis indicated that at the high concentration neutrophils and eosinophils increased with a concomitant decrease in macrophages. Ulceration of the nasal cavity was observed in males and females in the high concentration group. In males, there was an increase in cell count and total protein across all doses. In females, there was an increase in LDH across all concentrations, but the small sample size precluded establishing statistical significance for the effects. Minimal to mild increased mucus of the respiratory epithelium was observed in males and females at all concentrations. A conservative LOAEC of 0.08 mg/m^3 was identified based on increased mucus of the respiratory epithelium and increased LDH could be established for these effects; however, due to the mild effects and low number of animals/group, the effects were not statistically significant.

In the 13-week sub-chronic study, male and female Sprague-Dawley rats (10/group/sex) were exposed in whole body exposure chambers to concentrations of 0.11, 0.36, and 1.41 mg/m^3 (Kim et al., 2017). The MMAD of the DDAC aerosol was 0.63-1.65 µm, and the GSD was 1.62-1.65 µm. Body weight was confirmed to be clearly influenced by exposure to DDAC and mean body weight was approximately 35% lower in the high (1.41 ± 0.71 mg/m^3) male group and 15% lower in the high (1.41 ± 0.71 mg/m^3) female group compared to that of the control group. Albumin and lactate dehydrogenase were unaffected in the BALF. Lung weight was increased in females in the mid- and high-concentration groups in females and in males in the high concentration group only, which was accompanied by inflammatory cell infiltration and interstitial pneumonia in the mid- and high-concentration groups. Tidal volume and minute volume were not significantly affected at any concentration. Severe histopathological symptoms such as proteinosis and/or fibrosis, were not reported. A NOAEC of 0.11 mg/m^3 was identified based on the increased lung weights in females and increase in inflammatory cells.

BAC – benzalkonium chloride

BAC was evaluated in a 2-week whole-body inhalation study in male and female Fischer rats (5/group/sex) to concentrations 0.8, 4 and 20 mg/m^3 (Choi et al., 2020). The MMAD of the aerosols was 1.09-1.61 µm and the GSD was 1.51 to 2.00 µm. More exposure-related effects were observed in the upper airway. Nasal discharge, rale, and deep respiration were observed in the high dose group, and nasal discharge was observed in the low and mid dose groups. In the nasal cavity, ulceration with suppurative inflammation, squamous metaplasia, and erosion with necrosis were observed in the respiratory epithelium and transitional epithelium of the male and female high dose groups.

Degeneration and regeneration of terminal bronchiolar epithelium, smooth muscle hypertrophy of bronchioloalveolar junction, and cell debris in the alveolar lumens was observed in the mid and high dose male groups and high dose female group. Hypertrophy and hyperplasia of mucous cells in the bronchi or bronchiole were observed in both males and females. The authors hypothesized that BAC has greater deposition to the upper respiratory tract due to mucociliary clearance and emergency airway response caused by the irritation of BAC. The squamous metaplasia of the respiratory epithelium and transitional epithelium, mucinous cell hypertrophy and proliferation of the respiratory epithelium, mucinous cell metaplasia of the transitional epithelium in the nasal cavities, and mucinous cell hypertrophy and proliferation of terminal bronchiole which were observed in the study were considered adaptive changes after tissue injury. In the BALF analysis, the concentration of ROS/RNS, IL-1β, IL-6, and MIP-2 decreased dose dependently at the end of the exposure period but did not show a concentration-dependent change at 4 weeks of recovery. In addition, the concentrations of TNF-α, IL-4, and TGF-β did not show changes associated with test substance exposure. Finally, relative lung weights were statistically significantly increased in males at the mid and high doses and in females at the high doses only. The study authors concluded a LOAEC of <0.8 mg/ m^3 based on effects in the nasal cavity.

Mechanistic studies

Effects of cationic surfactant BAC on cell viability, inflammatory response and oxidative stress of human alveolar epithelial cells cultured in a dynamic culture condition were studied (Jeon, Haejun, et. al., 2019). To reflect the natural microenvironment of the lung, particularly its dynamic nature, the authors simulated normal breathing levels (tidal volume 10%, 0.2Hz) through surface

elongation of an elastic membrane in a dynamic culture system. This type of dynamic system provided easy control of breathing rate during lung cell culture. The system assessed the toxicity using different BAC concentrations (0, 2, 5, 10, 20, and 40 µg/mL) under static and dynamic culture conditions. Following 24 hr exposure to BAC, cellular metabolic activity, interleukin-8 (IL-8) and reactive oxygen species (ROS) levels demonstrated significant differences when using either static or dynamic cell growth conditions. The dynamic culture system, which more closely mimics lung conditions, showed higher toxic response to BAC.

Dose-Response Analysis: Quantitative Points of Departure (PODs)

Commented [HT29]: calculation of the HEC through application of a DAF is considered to address the toxicokinetic but not the toxicodynamic component of the animal-tohuman extrapolation.

The fairly limited animal inhalation toxicity data identified by the literature search and PODs from the studies reviewed summarized in Table **Y**. All of the identified data are from animal studies and therefore need to be extrapolated to estimate the human inhalation exposure (EPA, 1994). Previously, the exposure duration adjustment was described. EPA has also developed guidance focused on improving the science underlying the animal-to-human uncertainty factor provides generalized procedures for deriving dosimetric adjustment factors (DAF) (EPA, 1994; 2002). Application of DAFs to the animal airborne exposure values yields estimates of the concentration that would result in the same concentration to humans, that is, the Human Equivalent Concentration (HEC). Application of a DAF in the calculation of a HEC is considered to address the toxicokinetic aspects of the animal-to-human UF (i.e., to estimate from animal exposure information the human exposure scenario that would result in the same dose to a given target tissue) (EPA, 2002). This procedure involves the use of species-specific physiologic and anatomic factors relevant to the form of pollutant (*e.g.*, particle or gas) and categorized with regard to elicitation of response . These factors are all employed in determining the appropriate DAF. For

Commented [HT30]: Maybe this goes in the TSCA methods section? Or, because its 'new' to this category, put here?

HECs, DAFs are applied to the "duration-adjusted" concentration to which the animals were exposed (e.g., to a weekly average). The generalized DAF procedures may also employ chemical-specific parameters, such as mass transport coefficients, when available.

The Regional Deposited Dose Ratio (RDDR) was used to derive DAFs for each of the surfactants with available animal toxicity studies. The RDDR is the ratio of the deposited dose in a respiratory tract region (r) for the laboratory animal species of interest (RDD_A) to that of humans (RDD_H) and was derived according to EPA's "*Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry*" (EPA, 1994). EPA's RDDR software allows calculation of calculate RDDRs in various regions of the respiratory tract for animals versus humans (*i.e.*, extra-thoracic, tracheobronchial, pulmonary, thoracic, total respiratory tract and extra-respiratory regions). The RDDR calculation is based on the characteristics of the aerosol tested in the inhalation study (Median Mass Aerodynamic Diameter or MMAD, Geometric Standard Deviation or GSD), animal species, animal mass, gender, etc. The RDDR selected as the DAF is informed by the effects (clinical signs, tissue effects, biochemical changes) observed in the animal toxicity study and the aerosol characteristics in the inhalation study. The summary of RDDR inputs (*e.g.*, MMAD and GSD) and results are provided in Table Y for each of the toxicity studies from which PODs could be identified.

For the nonionic surfactant, Oxynonal 9 (Triton-X 100), the effects observed (increased lung weights, alveolar/bronchiolar epithelial hyperplasia and lung inflammation) are consistent with lung effects in the LRT such that the pulmonary region RDDR (0.564) was used to calculate the HEC. For the anionic surfactant, oleoylsarcosine, the effects were seen in multiple regions of the respiratory tract, including squamous metaplasia and epithelium proliferation and submucous

acute inflammation at the base of the epiglottis and early stages of fibrosis in the alveoli walls. Therefore, total respiratory tract RDDR (1.504 for males and 0.970 for females) was used to calculate the HEC. In both 21- and 90-day inhalation studies with DDAC, effects observed (changes in BALF LDH, BALF total protein, BALF cell count (males only), increase in mucus in the respiratory epithelium, increase in hemorrhage, and increase in mucoid exudate, inflammatory cell infiltration and interstitial pneumonia) were indicative that the pulmonary RDDR (0.42 for 21-day exposure and 0.5 to 0.6 for 90-day exposure) is appropriate for calculating the HEC. In contrast, for the cationic surfactant, benzalkonium chloride histopathological cellular changes were observed in the nasal cavity and lungs, indicating the total respiratory tract RDDR should be used to calculate the HEC. The RDDRs applied and HECs derived from the animal study PODs are provided in Table Y.

TABLE Y HERE – SEE SEPARATE FILE

Benchmark Margin of Exposure Analysis

The analogues shown in Table X provide representative examples of the types of PODs that may be applied to new chemistries that meet the Surfactant Criteria. Though the initial starting point for deriving a benchmark MOE is based on a composite of the default values of 10 for each of the individual values for UF_H, UF_A, and UF_L, refinements may be warranted based on dosimetric adjustments to the applied concentrations used for establishing the experimental PODs. As shown in Table Y, the data-derived uncertainty factors, RDDRs were used as DAFs to account for animal-to-human toxicokinetic difference.

In the case of surface-active substances like chemical substances meeting the Surfactant Criteria, EPA has recently adopted a generalized approach that has historically been applied on a case-by-case basis for chemical substances, in recognition that surface-active effects that lead to irritation/corrosion do not require absorption, metabolism, distribution, or elimination (ADME) (EPA, 2019). In the context of this publication, irritation/corrosion include those effects in the respiratory tract that lead, for example, to inflammation, hyperplasia, and metaplasia. For chemical substances that act *via* a surface-active adverse outcome pathway (AOP), the default values for UF_H and UF_A are reduced to 3 (*i.e.*, $10^{0.5}$ or 3.162) to account for the uncertainty/variability for toxicodynamics, whereas the toxicokinetic component is reduced to 1 because ADME differences that would otherwise influence toxicokinetic differences are generally not relevant for surface-active substances. In order to apply these reductions, the following criteria must be established:

Commented [HT31]: Need Citation at end of this paragraph...assume it is the OPP Guidance??

1. A description of the AOP,
2. A discussion of why the AOP is unlikely or likely to differ between humans, in the case of UF_H, or between animals, in the case of UF_A, and
3. A discussion as to why the ADME of the chemical substance is unlikely to play a role in the observed toxicity.

When the above criteria are met, application of the appropriate dosimetric adjustment factor (*i.e.*, RDDR) should still be applied, given that deposition is the most appropriate dosimetric for assessing acute/subacute effects from surface-active agents. However, when dosimetric adjustments are applied, the reduction in the toxicokinetic component for UF_A are subsumed by the overall reduction, that is, no additional reductions should be incorporated.

Based on these information and criteria, the following composite values are appropriate to describe intra- and interspecies uncertainty/variability (*i.e.*, $UF_H \times UF_A$):

UF_H = 10 or 3: The default value of 10 should be applied when the available information does not support each of the above criteria. If the available information supports all of the above criteria, then a value of 3 may be applied.

UF_A = 10 or 3: The default value of 10 should be applied when the available information does not support the application of a dosimetric adjustment factor to quantifying a human equivalence concentration (HEC) or when the available information does not support each of the above criteria. If the available information allows derivation of an HEC and/or application of the above criteria, then a value of 3 may be applied.

UF_L = 10 or 1: If the POD from the experimental study is based on a LOAEC, then a default value of 10 should be applied, unless there is information to support that a reduced value is warranted. If the experimental data are amenable to benchmark dose modeling, a BMCL should be calculated and a value of 1 should be applied for this area of uncertainty.

Taken together, the above considerations and approaches support application of a benchmark MOE ranging from 10 to 1,000 and will depend on the analogue used and available data on the new chemical substance. In those instances where the data are too limited to determine when an analogue is appropriate for extrapolating the hazards to the new chemical substance,

experimental testing should be performed to aid with informing the quantitative assessment, as discussed under the Tiered-Testing Strategy.

Uncertainties and Limitations

The assessment framework outlined herein includes a number of uncertainties and limitations, include those associated with extrapolating the hazards identified from the analogues shown in shown in Table Y. Uncertainties associated with using animal studies to estimate human toxicity are recognized and methods developed to reduce them (OECD, 2014). Exposure duration adjustment procedures for inhalation exposures and application of DAFs to derive HECs, are well-established procedures for reducing uncertainties associated with the toxicokinetic aspects of animal-to-human extrapolation (EPA, 1994; EPA 2002). factors and derivation of benchmark MOEs (*i.e.*, type and magnitude of uncertainty factors). Likewise, EPA has recommended that BMD modeling be employed whenever possible to identify a POD and to reduce uncertainties associated with using a LOAEL from a toxicity study.

Commented [ST32]: OECD, 2014. [HYPERLINK "https://gcc01.safelinks.protection.outlook.com/?url=http%3A%2F%2Fwww.oecd.org%2Fofficialdocuments%2Fdisplaydocument%2F%3Fcote%3Denv%2Fjm%2Fmono(2014)4%26doclanguage%3Den&data=02%7C01%7CStedeford.Todd%40epa.gov%7C283d690eae994f6079e908d82dae913d%7C88b378b367484867acf976aacbeca6a7%7C0%7C0%7C637309575062395679&sdata=9%2BoEl9lB15HrNbOxTYXxIUBmTOrRyO5lCq4uT4rOiAM%3D&reserved=0" \t "_blank"], second edition Series on Testing and Assessment No. 194, 2014

[HYPERLINK "https://gcc01.safelinks.protection.outlook.com/?url=https%3A%2F%2Fwww.oecd.org%2Fenv%2Fehs%2Frisk-assessment%2Fgroupingofchemicalschemicalcategoriesandread-across.htm&data=02%7C01%7CStedeford.Todd%40epa.gov%7C283d690eae994f6079e908d82dae913d%7C88b378b367484867acf976aacbeca6a7%7C0%7C0%7C637309575062400652&sdata=RKUKYR%2FGjw%2FOunS0Tg9CIA2m4KqTzS%2BWoahkuxLHz6o%3D&reserved=0"]

Given the small number of chemical substances that meet the Surfactant Criteria that have concentration-response inhalation toxicity data, the applicability of these analogues to new chemical substances needs to be carefully considered, particularly given the influence of additional functional groups that may increase/decrease the toxicity of the new chemical substance compared to the comparator analogue. Risk assessors should first consider the surface tension and CMC criteria provided in Table X, and compare them to these measurements for the new chemical substance, if available, or the influence additional functional groups present or absent from the new chemical would have on these criteria (*e.g.*, would a particular functional group increase or

Commented [HT33]: This is where WI's comment re: predicting potency, if turned into actual explanation with citations, could be useful.

decrease hydrophobicity or hydrophilicity and thereby increase or decrease CMC?). If such structural differences are judged not to significantly influence properties and toxicity, such that the new chemical substance is expected to have comparable or lower toxicity, read-across is an appropriate approach for characterizing hazards and risk. Of course, uncertainties regarding read-across should be acknowledged in the risk characterization.

For instances where the notifier of the new chemical substance and/or EPA is unable to conclude that one of the analogues in Table Y is comparable to or represents a worse-case analogue compared to the new chemical substance, then the Tiered-Testing Strategy provided herein should be employed to inform whether the new chemical substance has lower, comparable, or higher toxicity to the most representative analogue in the respective subcategory. Prior to conducting such testing, the scientific basis for selecting an analogue as the comparator compound to the new chemical substance should be understood and a rationale provided as to why the analogue is anticipated to have comparable or higher toxicity than the new chemical substance.

Commented [ST34]: William comment: "Surface tension and p-chem data may be able to rank the potency of the surfactants within a group."

Commented [HT35R34]: Tell us how? Describe the RELATIONSHIP, E.G. increase in ST would increase/decrease toxicity and increase/decrease in CMC is correlated with increases/decreases tox...are there papers on these relationships?

Use of New Approach Methods (NAMs) and *In Vitro* Testing Strategies to Avoid Excessive Animal Testing

The amended TSCA requires EPA to reduce reliance on animal testing using methods and strategies that "provide information of equivalent or better scientific quality and relevance for assessing risks of injury to health or the environment" (EPA, 2016). Additionally, in 2019, EPA wrote a directive to prioritize efforts to reduce animal testing by using NAMs (Wheeler, 2019). Multiple NAMs exist which can be used to assist in the hazard and risk assessment of new chemical substances that meet the Surfactant Criteria, including validated OECD methods for *in*

vitro irritation testing, as well as new *in vitro* methods to specifically assess respiratory toxicity. While several of the methods are described below, it is understood that this field is quickly advancing. Therefore, additional NAMs that are not described below may be discussed with EPA during a pre-notice consultation meeting.

Surfactants are proposed to cause a specific sequence of biological events in the pulmonary region if they are manufactured or used in a respirable form (*i.e.*, $\leq$ 10 µm). Therefore, an initial consideration of the potential for a surfactant to cause pulmonary toxicity is whether it is respirable. Several validated methods exist for making this determination (*e.g.*, cascade impactor, laser methods, OECD TG 110 and OPPTS 830.7520). As a practical matter, we propose using a cutoff of > 1% respirable particles/droplets by weight (wt%) for data obtained with these assays on the surfactant and/or a mixture containing the surfactant. This cutoff is consistent with EPA's "trace amounts" threshold for the nonreportable content for nanoscale materials (EPA, 2017).

If a surfactant is respirable, the next step with evaluating its potential to cause pulmonary toxicity would typically be *in vivo* inhalation assays; however, one approach for utilizing non vertebrate testing methods includes establishing a framework of events called an AOP. An AOP is an analytical construct that describes a sequential chain of causally linked (key) molecular or cellular events that lead to an adverse health effect that affects the organism and provides key information that may be used for informing quantitative risk assessment without the use of data obtained from vertebrate animals or, at a minimum, reducing the types of vertebrate animal data needed.

AOPs are the central element of a toxicological knowledge framework being built to support chemical risk assessment based on mechanistic reasoning (Leist et al, 2017). Representative key elements of AOPs are the molecular initiating events (MIEs), cellular level events (CLEs), organ or tissue level events (OLEs), and organism consequent events (OCEs). For surfactants, the crucial initial key event is proposed to be the interaction of the substance with lung-surfactant (MIE) and/or the molecular interaction of the substance itself with cell membranes (MIE), resulting in the disruption of lung cells due to loss of lung cell surfactant function (CLE) and/or the loss of membrane integrity (CLE). These initial events may lead to different OLEs (*e.g.*, alveolar collapse, loss of barrier function, blood extravasation, and impaired oxygenation of blood), which may finally lead to organism consequences (OCE) such as e.g. pneumonia, limited lung function by chronic obstruction (COPD), fibroses, etc.

Commented [KA36]: Arch Toxicol . 2017 Nov;91(11):3477-3505. doi: 10.1007/s00204-017-2045-3.

In vitro tests, such as by capillary surfactometer, may be useful in preliminary screening of chemicals to be tested, but do not by themselves constitute adequate tests for acute pulmonary effects of these chemicals. Therefore, if comparable concentrations are used in *in vitro* models, there will be a probability to get an overprediction in the results. This information should be taken into consideration within the design of additional *in vivo* tests.

Commented [KA37]: I am not sure what is meant here. Needs rewording by author.

Commented [SK38]: This belongs in the NAMs section not hazard ID. I've moved.

In vitro systems may help to investigate specific key events in the AOP and confirm that the substance may act like a typical surfactant (group assignment *via* similar AOP) and/or if other substance specific properties lead to a predominant type of key events within the AOP. Further, *in vitro* tests may also deliver information for avoiding *in vivo* testing (*e.g.*, corrosive substances cannot be tested due to animal welfare reasons) or providing helpful information on dose selection for *in vivo* testing, if needed. These assays can be used as part of a weight of scientific

evidence evaluation under Section 26(i) of TSCA, to determine whether animal testing is needed or if a point of departure (POD) can be determined for risk assessment purposes without the use of animals. These tests may also provide insight on the AOP.

Based on the AOP framework above, a number of different types of *in vitro* test methods, summarized in Table XX, may provide potentially useful information for informing the various elements of the surfactant AOP.

Table XX. *In Vitro* Test Methods That May Be Useful for Evaluating the AOP for Lung Effects of Surfactants.

Surfactant AOP	*Information on AOP*	*In Vitro* Assay	Test System
MIEs	MIE for interaction with pulmonary surfactant/loss of function	Specific *In Vitro* Respiratory Toxicity Assays	• *In vitro* lung surfactant inhibition as described by Sorli et al., (2017)
	MIE for interaction/penetration through cell membrane	*In Vitro/Ex Vivo* Irritation Assays	• OECD *In vitro/Ex Vivo* eye irritation tests for penetrance, *e.g.*: (OECD 492) Reconstructed human Cornea-like Epithelium (RhCE), (OECD 437) Bovine Corneal Opacity and Permeability Test, (OECD 438) Isolated Chicken Eye Test, *etc*.
CLEs	CLE for loss of membrane integrity/general cytotoxicity	*In Vitro/Ex Vivo* Cytotoxicity Assays	• OECD *In vitro/Ex Vivo* eye irritation tests for cytotoxicity, *e.g.*: (OECD 492) Reconstructed human Cornea-like Epithelium (RhCE), (OECD 437) Bovine Corneal Opacity and Permeability Test, (OECD 438) Isolated Chicken Eye Test, *etc*. • Cell membrane integrity test (LDH-lactate dehydrogenase cytotoxicity assay), MTT assay or lysosomal membrane integrity test. • BALB/c3T3/A549 lung cells neutral red uptake (NRU) cytotoxicity test, a test for basal cytotoxicity [HYPERLINK "https://ntp.niehs.nih.gov/iccvam/docs/acutetox_docs/brd_tmer/at-tmer-complete.pdf"]
OLEs	OLE for tissue level events	Human organotypic airway epithelial cultures	• EpiAirway™ 3-D constructs of human-derived cell cultures of differentiated airway epithelial cells • MucilAir EpiAirway™ 3-D constructs of human-derived cell cultures of differentiated airway epithelial cells
	OLE for tissue level events	Specific *Ex Vivo* Respiratory Toxicity Assays	• Precision-cut lung slice test *etc*. as described by Hess et al (2016)

[PAGE]

MIEs

The surfactant AOP is assumed to consist of two MIEs that may be informed by *in vitro* assays to determine whether a particular chemistry causes adverse effects on the pulmonary surfactant system (MIE #1), pulmonary cell membranes (MIE #2), or both. For MIE #1, Sorli et al., (2017) developed an *in vitro* lung surfactant inhibition assay that specifically measures whether the substance interferes with lung surfactant function. The assay was initially benchmarked for predicting the effect of waterproofing agents that were shown to be acutely toxic to mice. The authors noted that it may be overly conservative for some substances. Nevertheless, this assay investigated a basic principle (MIE #1) which may also be relevant for some types of surfactants. For MIE #2, the *in vitro* eye irritation assays represent appropriate screening approaches for determining the ability of surfactants to interact with cellular membrane and penetrate through the corneal layer of the eye. For example, Bader et al., (2013) showed that the BCOP assay was effective at identifying the potential for nonionic (*i.e.*, Triton X-100), anionic (*i.e.*, SDS), and cationic (*i.e.*, benzylalkonium chloride) substances to cause irritation to the eye; however, the authors also noted that the endpoints evaluated in this assay should be carefully assessed independently. For Triton X-100 and SDS, the permeability score was more predictive of eye irritation than the ocular opacity score, whereas for benzylalkonium chloride, the opacity score was more predictive of eye irritation than the permeability score. Therefore, a systematic investigation with surfactants using this approach may be helpful with elucidating MIE #2 of the AOP. In addition, information on the potential of a substance to cause *in vitro* skin irritation (e.g. OECD TG439) and/ or *in vitro* skin corrosion (OECD TG 431, when available, can provide orthogonal evidence of the potential for a substance to cause similar irritant or corrosive effects

in respiratory tract cells. Importantly, substances that are found to be corrosive cannot proceed to *in vivo* testing due to animal welfare concerns. If the substance is found to be a severe irritant, subsequent *in vivo* testing, if warranted, should be designed to avoid severe irritation effects in animals. For example, acidic or alkaline substances can be pH-adjusted to neutral values to prevent pH-mediated irritation to animals during testing. Corrosion effects mediated by pH extremes should be distinguished from necrosis effects *via* membrane disruption, for example DDAC causes tissue effects in inhalation studies despite having a neutral pH value of 6.8-6.9 ([HYPERLINK "https://www.sigmaaldrich.com/MSDS/MSDS/DisplayMSDSPage.do?country=US&language=en&productNumber=34466&brand=SIAL&PageToGoToURL=https%3A%2F%2Fwww.sigmaaldrich.com%2Fcatalog%2Fproduct%2Fsial%2F34466%3Flang%3Den"]).

Commented [ST39]: William comment: "Corrosion can be due to acidity, alkalinity or the inherent ability to cause cellular necrosis. Alkaline or acidic compounds can be pH adjusted to neutral values."

Commented [ST40]: Todd to add to EndNote file

CLEs

Several *in vitro/ex vivo* assays are available that may aid with informing CLEs on general cytotoxicity in the surfactant AOP. For general cytotoxicity, the ocular irritation/corrosion studies cited in Table XX provide one set of options using cell types that are known to be sensitive to the effects of surfactants. Further, the NRU test has a validated protocol by ICCVAM using the BALB/c3T3/A549 lung cells, so there are test acceptance criteria, potential modifications for volatile substances, and stopping rules (for insoluble substances) (ICCVAM Test Method Evaluation Report, 2006). In each assay, surfactants with inhalation toxicity data such as Triton-X 100 and benzylalkonium chloride may be used as positive controls to

benchmark the results, thereby reliable results for estimating the potential for surfactants to cause irritation and cytotoxicity.

OLEs

Commented [ST41]: Note, the SmallAir system should be added to the above table, as possible OLE test systems

Based on the results of the testing on the CLEs, it may be necessary to perform more robust testing, given the limitations of these assays. For example, the discussed assays measure single cell types, whereas human and animal airway epithelia are composed of multiple cell types that each have specialized functions. Several human airway models have been developed that allow for the assessment of multiple endpoints in three-dimensional culture systems. Two commonly employed systems include EpiAirway™ and MucilAir™ developed by MatTek Life Sciences and Epithelix, respectively, and are discussed below.

Organotypic airway epithelial cultures, such as EpiAirway™ and MucilAir™, provide a more physiological *in vitro* model system compared to *in vitro* cell lines (EPA, 2018). Unlike single cell lines, these organotypic cultures take on a pseudostratified morphology, develop tight junctions, differentiate into multiple cell types, including: basal cells, ciliated cells, and goblet cells; generate mucus, exhibit ciliary beating, have xenobiotic metabolizing capacity, and maintain cultural homeostasis for months. Because of these characteristics, the human airway models are expected to better represent the response of *in vivo* tissue to surfactant exposure than cell line cultures of a single cell type. Depending upon the level in the respiratory system where the site of contact / exposure is predicted to occur, using for example MPPD modeling for determining deposition, different 3D cell culture systems are available that are composed of the different cell types that occur at different anatomical sites in the respiratory tract. For example,

Commented [KA42]: Issue Paper
Evaluation of a Proposed Approach to Refine Inhalation Risk Assessment for Point of Contact Toxicity:
A Case Study Using a New Approach Methodology (NAM)
EPA's Office of Chemical Safety and Pollution Prevention
August 30, 2018

MucilAir™ provides 3D co-culture models of cells from nasal, tracheal or bronchial sites, as well as a co-culture of cells from small airways (SmallAir™). EpiAirway™ is composed of normal human tracheal/bronchial epithelial cells as a co-culture system with normal human stromal fibroblasts, and EpiAlveolar™ is a 3D co-culture model of the air-blood barrier produced from primary human alveolar epithelial cells, pulmonary endothelial cells and fibroblasts.

Exposure to aerosols at the ALI using a Vitrocell® exposure system is a lower throughput approach to *in vitro* two-dimensional exposure systems; however, it provides a more comparable exposure to real-life exposure scenarios for inhaled aerosols. Using ALI exposure, dilution into medium and interaction with medium components does not occur as it would in a submerged culture system. There is interaction of the aerosol with a mucus or surfactant layer if organotypic cultures are used, as there would be *in vivo*, thus more physiologically relevant.

Exposures of these organotypic cultures at the ALI can be combined with a number of assays for assessing cell function and viability. Measurement of transepithelial electrical resistance (TEER), LDH-release, and viability assays such as MTT or ATP assays have all been reported for use with these cultures. These assays are multiplexable on the same cultures. TEER measures epithelial integrity, including functionality of intercellular tight junctions. LDH-release measures loss of plasma membrane integrity, which is indicative of cytotoxicity, and MTT and ATP assays measure cell viability. MatTek Life Sciences recommends the MTT assay for use with their EpiAirway™ cultures and recommends the surfactant Triton X-100 at 0.2% concentration as a

positive control for cytotoxicity. These assays can also be used to determine an HEC, which may be used for quantitative risk assessment.

While significant progress has been made toward achieving the objectives to use of high-throughput in vitro assays and computational models based on human biology to evaluate potential adverse effects of chemical exposures (NAS 2007, NAS 2017), the investigation of effects using *in vitro* models of higher levels of biological organization remains challenging. All other things being equal, for relevancy to humans and for animal welfare considerations, the 3D human airway cell culture systems discussed above would be the test systems to be aspired. However, depending on a number of factors, including the type of substance and specific decision context, use of different alternative assays may be considered. For example, the precision-cut lung slice (PCLS) test measures multiple endpoints, such as LDH for cytotoxicity and IL-1α for pro-inflammatory cytokine release in *ex vivo* cultures of rodent lung slices, to determine whether a chemical is likely to be toxic to the respiratory tract by inhalation exposure (Liu et al., 2019).

Commented [RAB43]: NAS 2007 Toxicity Testing in the 21st Century [HYPERLINK "https://www.nap.edu/catalog/11970/toxicity-testing-in-the-21st-century-a-vision-and-a"]

NAS 2017 Using 21st Century Science to Improve Risk-Related Evaluations [HYPERLINK "https://www.nap.edu/catalog/24635/using-21st-century-science-to-improve-risk-related-evaluations"]

Commented [RAB44]: Liu et al. 2019 [HYPERLINK "https://respiratory-research.biomedcentral.com/articles/10.1186/s12931-019-1131-x"]

PCLS contain intact alveoli, rather than monolayers of one or two cells types (co-cultures). Crucially, in contrast to organoids, cell types are present in the same ratios and with the same cell–cell and cell–matrix interactions as *in vivo*. PCLS are often utilized in toxicological and anatomical studies regarding contractility in relation to asthma and other respiratory illnesses, such as emphysema (Sanderson et. al. 2011). Therefore, physiological responses, other than cytotoxicity, that may be evoked by the surfactant may be monitored. One further advantage of PCLS is that the PCLS assay can be performed on multiple species to determine susceptibility.

Commented [SM45]: Michael J. Sanderson, Ph.D. Exploring lung physiology in health and disease with lung slices
Pulm Pharmacol Ther. 2011 October ; 24(5): 452–465.

The PCLS test system has been pre-validated in multiple, independent laboratories, and the results showed good correlation when translated from *in vivo* LC_{50} values (Hess et al., 2016). While this assay has not yet been systematically used for surfactants, it may be considered for such substances once a solid database is established. While considered an alternative test, this assay still requires use of laboratory animals, albeit that, compared to *in vivo* inhalation tests, this assay reduces the number of animals that would be needed to conduct dose response studies. From a rat lung (1 g), about > 200 slices can be prepared. In general, for 1 concentration, 2 slices are used, resulting in 100 different concentrations or repeats that can be tested with one sacrificed rat. Additionally, PCLS cultures are stable for up to 4 weeks and allows for exposures via media or air with additional adaptations. The PCLS system can be considered to be an additional tool in the inhalation toxicity assay tool box. The rationale for selection of the PCLS assay, as with any inhalation toxicity assay, should be scientifically justified in advance of initiating testing.

Uncertainties/Limitations

The previous assays discussed under each of the respective surfactant AOP elements (*i.e.*, MIEs, CLEs, and OLEs) represent assays that may inform the potential inhalation toxicity from these substances; however, there are several uncertainties/limitations with these assays that warrant discussion. Though some of these are discussed elsewhere for each of the above testing systems, as well as others (Clippinger et al., 2018), it is important to consider that these assays were not systematically tested using surfactants and benchmarked against *in vivo* inhalation toxicity data on surfactants. Though we have recommended specific assays for evaluating the surfactant AOP,

a priori to using any or all of these tests is whether they can provide data that are comparable to *in vivo* tests and are suitable and fit for purpose in quantitative risk assessment.

In this regard, approaches to evaluate the scientific confidence of test methods for hazard assessment and risk assessment have, and continue to, evolve. A fit for purpose framework, employing specific criteria to establish relevancy, reliability, variability, sensitivity, domain of applicability, *etc*., for evaluating and documenting the scientific confidence of a new method for use for informing specific decision context has emerged from the regulatory science community to address the challenges posed for validation of NAMs that provide scientific rigor, but that are also flexible and adaptable (Parish et al., 2020; Patlewicz et al., 2015, EPA 2020).

Commented [RAB46]: https://www.sciencedirect.com/science/article/pii/S0273230020300180
[HYPERLINK "https://www.sciencedirect.com/science/article/pii/S0273230020300180"]
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[HYPERLINK "https://www.epa.gov/sites/production/files/2020-06/documents/epa_nam_work_plan.pdf"]

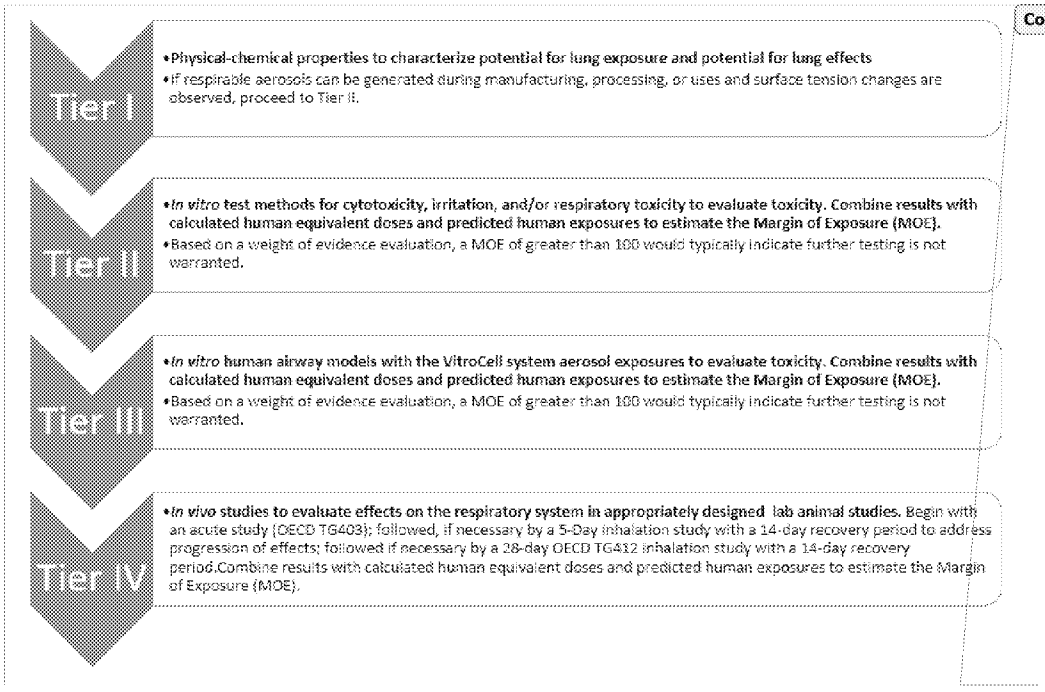
Once such fit for purpose scientific confidence evaluations are documented, there are several ways that these assays can be used to avoid excessive animal testing. First, testing can be performed on the surfactant AOP to evaluate the potency of new surfactants versus a comparator surfactant (*i.e.*, positive control) within the relevant subcategory that has repeated concentration inhalation toxicity data. Second, depositional data using models such as RDDR or MPPD for determining the depositional fraction of the new surfactant may be used for test concentration estimation and for estimating a potency ratio. Finally, *in vitro* to *in vivo* extrapolations (IVIVEs) may be used to determine a HEC for quantitative risk assessment.

Commented [OS47]: Tala to include some additional text – read across, etc.

Tiered-testing Strategy

An approach to tiered testing is presented in Figure 1 and discussed in detail below. Drawing from the assays discussed above (and summarized in Table XX), this tiered testing and evaluation approach commences with the least complex, most efficient testing method, and then, at each subsequent tier, the complexity of the test system increases to more effectively emulate the biology and physiology of the *in vivo* respiratory tract system.

Draft Figure 1.



Commented [OS48]: Additional changes coming

Tier I—Physical-chemical properties

- Particle size distribution or aerosolized droplet size (*i.e.,* cascade impactor, laser methods) (OECD TG 110, Office of Prevention, Pesticides and Toxic Substances [OPPTS] 830.7520, OECD Guidance Document [GD] 39).

If respirable particles/droplets can be generated at greater than 1 wt% during manufacturing, processing, or any of the uses for the new chemical substance, proceed to Tier II.

Commented [OS49]: Raphael: As per polymer overload, having a mg/m3 metric in addition to the 1% respirable would be helpful in certain situation e.g. very low particle/droplet emission during use so measuring 1% respirable is technically challenging or not feasible.

Commented [ST50R49]: I need to discuss this with Tala. The mg/m3 approach for this category is a bit more complicated than for the PLO category.

Tier II—*In vitro/Ex vivo* studies

The following *in vitro/ex vivo* test methods may provide potentially useful information towards with informing MIEs and CLEs. In order to determine the best approach for *in vitro*/*ex vivo* testing, a pre-notice consultation with EPA should be considered, given that none of the following studies are validated to determine lung toxicity induced by surfactants. In general, the testing approach should include a combination of assays, such as one on "Pulmonary surfactant interaction/loss of function", one on "Cell interaction/penetration", and one on "General cytotoxicity". The *in vitro*/*ex vivo* eye irritation studies may satisfy the latter two endpoints. If equivocal findings are obtained on the "Cell interaction/penetration" or "General cytotoxicity" assays, then the NRU cytotoxicity test should be performed. For each assay, the representative analogue to the new chemical substance for the respective subcategory of surfactants should be used as a positive control. Further, dosimetry models such as RDDR or MPPD should be used to simulate human exposures and to aid with identifying the appropriate test concentrations for the *in vitro*/*ex vivo* test systems, considering for example the surface area of the culture system or *ex vivo* tissue, loss mechanisms, *etc*.

Pulmonary surfactant interaction/loss of function

- *In vitro* lung surfactant inhibition as described by Sorli et al., (2017)

Cell interaction/penetration

- OECD *In vitro* eye irritation tests, *e.g.*: (OECD 492) Reconstructed human Cornea-like Epithelium (RhCE), (OECD 437) Bovine Corneal Opacity and Permeability Test, (OECD 438) Isolated Chicken Eye Test, *etc*.

General cytotoxicity

- OECD *In vitro* eye irritation tests, *e.g.*: (OECD 492) Reconstructed human Cornea-like Epithelium (RhCE), (OECD 437) Bovine Corneal Opacity and Permeability Test, (OECD 438) Isolated Chicken Eye Test, *etc*.
- Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) recommended protocol for the BALB/c 3T3/A549 lung cells neutral red uptake (NRU) cytotoxicity test, a test for basal cytotoxicity (Appendix C1, [HYPERLINK "https://ntp.niehs.nih.gov/iccvam/docs/acutetox_docs/brd_tmer/at-tmer-complete.pdf"])

Each of the assays may be used to determine a starting point to calculate a modified POD_{HEC} using *in vitro* to *in vivo* extrapolation (IVIVE). The most sensitive of the endpoints identified from the assays should be used to calculate a POD using BMD modeling, when possible, with the $BMCL_{1SD}$ metric. This metric is based on the benchmark response (BMR) of one standard

deviation suggested for *in vitro* assays (a ~14.9% change from the control group value for the TEER assay), per the 2018 FIFRA Inhalation Scientific Advisory Panel meeting ([HYPERLINK "https://www.regulations.gov/docket?D=EPA-HQ-OPP-2018-0517"]). However, alternative metrics may be considered. For example, the pharmaceutical industry has utilized fixed adverse response thresholds that are appropriate for the specific biological assay (*i.e.*, EC_{15}, EC_{30}, *etc*; O'Brien 2006). Regardless of the metric used, a justification for its selection should be provided. ~~The *in vitro* POD can be converted to a deposited dose using the Multiple-Path Particle Dosimetry (MPPD) model for aerosols.~~ In those situations where data are not amenable to BMD modeling, due to assays that are not designed to provide concentration response data and/or lack sufficient granularity, the *in vitro* testing concentration level should be determined based on the expected HEC (taking into account the necessary MOE) to ensure that the *in vitro* data are generated in a concentration range relevant to the expected HEC. This alternative approach may be well suited when the expected human deposited dose is much lower than the typical/standard *in vitro* testing exposure dose.

Commented [ST51]: Note, I deleted this b/c of the statement above about using RDDR or MPPD for determining test concentrations.

When the data are amenable to calculating an HEC, the relevant routes of exposure should be considered, based on the conditions of use. An ~~margin of exposure~~MOE may then be determined by dividing the HEC by the estimated exposure and comparing to the benchmark MOE for the respective positive control.

Commented [RAB52]: I think this MOE sentence needs to be included to match up with the text in the tiered testing figure

Based on the results of the above testing combinations, the following outcomes are possible, noting that a positive result in one of the 3 assays, will drive the determination of "greater" or

Commented [RAB53]: Its not clear how MOE fits into these decision criteria. I inserted draft text below – highlighted -- as a suggestion – please review and revise as needed

"comparable" toxicity, whereas negative results in all 3 assays will drive the determination of "lower" toxicity, as described below.

If the new chemical substance exhibits greater toxicity to the positive control in one of the evaluated assays, per the study method criteria, proceed to Tier III.

If the new chemical substance exhibits comparable toxicity to the positive control, per the study method criteria, in one of the evaluated assays, then stop at Tier II. It may be necessary, depending on the ~~margin of exposures~~MOE for specific conditions of manufacturing, formulation, and use to consider engineering controls and/or appropriate PPE requirements for worker risks and/or reformulation of the new chemical substance at a lower wt% in products for consumer risks.

If the new chemical substance exhibits lower toxicity or negative findings relative to the positive control, per the study method criteria, in all the evaluated assays, then determine if a modified POD_{HEC} can be calculated from the representative analogue in the respective subcategory of surfactants. If a modified POD_{HEC} can be calculated, then recalculate the MOE ~~reassess risks~~ using the modified POD_{HEC}. ~~using MOE as the risk metric~~ If risks are still identified with the modified POD_{HEC}, then stop at Tier II and consider engineering controls and/or appropriate PPE requirements for worker risks and/or reformulation of the new chemical substance at a lower wt% in products for consumer risks. If it is not possible to calculate a modified POD_{HEC}, then proceed to Tier III.

Tier III – Human Airway Models/PCLS Assay

- ~~Mat-Tek and/or Epithelix 3D human airway cells with VitroCell system aerosol exposures~~

~~*In vitro* to *in vivo* extrapolation to develop a $_{HEC}$ in Tier III is similar to the approach pursued in Tier II. The margin of exposure will be calculated by dividing the $_{HEC}$ by the exposure. While the exposure will be the same between Tier II and III, some uncertainty factors regarding the $_{HEC}$ can be avoided as the ALI-based exposure is more consistent with inhalation exposure in a human than the submerged culture exposures employed in Tier II (EPA, 2018). For inhaled surfactants the AOP is expected to be related to the physical chemical properties of these substances leading to impacts on lung surfactant or cell membranes. Because these effects are related to the concentration at the site of contact in the respiratory tract, this AOP does not require the typical ADME considerations used for selecting uncertainty factors for systemic toxicants. Instead, a default adjustment factor of unity for interspecies extrapolation for local effects via this AOP is considered to be scientifically justified (ECETOC 2014 http://www.ecetoc.org/wp-content/uploads/2014/08/ECETOC-TR-110-Guidance-on-assessment-factors-to-derive-a-DNEL.pdf).~~

Several testing options are available for evaluating OLEs in the surfactant AOP. The test system employed should focus on evaluating effects in the respiratory tract at the predicted sites of deposition (e.g., TB and/or PU regions) using RDDR or MPPD modeling, as discussed previously. A justification for using a particular system(s) versus another should be provided and may be discussed with EPA as part of a pre-notice consultation. Available test systems include, but are not limited to, the following:

Commented [KA54]: Issue Paper
Evaluation of a Proposed Approach to Refine Inhalation Risk Assessment for Point of Contact Toxicity:
A Case Study Using a New Approach Methodology (NAM)
EPA's Office of Chemical Safety and Pollution Prevention
August 30, 2018

Commented [OS55]: Stay consistent AOP not MoA – search throughout

Commented [ST56R55]: I deleted this because it seems redundant with the Category benchmark MOE discussion.

Commented [ST57]: I deleted this because it doesn't appear relevant to our situation. The ECETOC document specifies that the reduction to unity is for gases and vapors, not aerosols. See p. 29 of the cited document.

- EpiAirway™ 3-D constructs of human-derived cell cultures of differentiated airway epithelial cells
- MucilAir EpiAirway™ 3-D constructs of human-derived cell cultures of differentiated airway epithelial cells
- Precision-cut lung slice test *etc.* as described by Hess et al (2016)

Based on the results of the 3D-construct and/or PCLS testing, *in vitro* to *in vivo* extrapolation may be possible for developing a POD_{BMC} for use with characterizing potential risks using the MOE approach. Though the occupational/consumer exposure estimates may be the same between Tiers II and III, the Tier III test results may offer the opportunity for refining the risk estimates. For example, the BMR used for calculating the POD_{BMC} may be refined because the ALI-based exposure is more consistent with inhalation exposure in a human than the submerged culture exposures employed in Tier II (EPA, 2018). Further, application of uncertainty factors for calculating the benchmark MOE may also be refined, if for example, human cultures are used, which may preclude the need for applying a UF_A.

If the Tier III test data are amenable for developing a POD_{BMC}, then the risk estimates should be reassessed. If no risks are identified under the conditions of use, then stop at Tier III. If risks are still identified under the conditions of use, then consider engineering controls and/or appropriate PPE requirements for worker risks and/or reformulation of the new chemical substance at a lower wt% in products for consumer risks.

Commented [ST58]: Note, the SmallAir system should be included here

Commented [KA59]: Issue Paper
Evaluation of a Proposed Approach to Refine Inhalation Risk Assessment for Point of Contact Toxicity:
A Case Study Using a New Approach Methodology (NAM)
EPA's Office of Chemical Safety and Pollution Prevention
August 30, 2018

If the Tier III test data are not amenable for developing a POD_{BMC}, then proceed to Tier IV.

~~A margin of exposure of greater than 100 may mean that *in vivo* testing is not warranted. Additionally, if certain uses are controlled so that exposure is not a concern, these uses could be approved, and additional uses could require SNUR. If not, then meetings with toxicology experts and EPA to discuss if further testing (*in vitro* or *in vivo*) is needed. Tier III and IV testing should only be done in consultation with EPA, and additional risk management options (e.g., engineering controls and personal protective equipment) should also be discussed. Even if additional *in vivo* testing is needed, these NAM assays can be used to determine a starting dose, potentially reducing animal testing.~~

Tier IV—*In vivo* studies

Strategic *in vivo* testing may be needed to inform the hazard and risk assessment of new chemical substances, particularly in those instances where a new chemical substance has unique properties that preclude a determination that one of the subcategory analogues is appropriate for read across, as well as in instances where the test data generated under Tiers II and III are not amenable for deriving POD_{BMDS}. If *in vivo* testing is needed, a pre-notice consultation meeting with EPA should be considered prior to initiating any testing.

~~Note that a prenotification consultation with EPA should be considered prior to undertaking any Tier IV testing.~~

The potential for surfactants to cause adverse effects on the respiratory tract are based on acute toxicity concerns, that is, interfering with pulmonary surfactant and/or disrupting cellular membranes. Since these effects may be captured using appropriate exposure concentrations in short-term inhalation studies, the following *in vivo* tests are recommended:

- Step 1: OECD Acute TG 403 (modified)** featuring rats exposed for 4 hours and observed for 2 weeks using aerosol testing. ~~As described above, the HEC should be derived using default or chemical-specific adjustment factors (CSAFs) and compared to potential actual human exposures to workers or consumers to determine a margin of safety or margin of exposure. Based on a weight of evidence evaluation in general, if the margin is >100, further testing is not needed.~~

- Step 2: 5-Day inhalation study with a 14-day recovery period** to address progression of effects (use OECD TG 412, but conduct exposure duration for at least 5 days). ~~Proceed to step 3 if study reports substantial decrease in the POD over time relative to the acute study, or if an increase in lung burden is observed. The HEC should be derived using default or chemical-specific adjustment factors (CSAFs) and compared to potential actual human exposures to workers or consumers to determine a margin of safety or margin of exposure. Based on a weight of evidence evaluation, in general, if the margin is >100, further testing is not needed.~~

~~* Step 3: OECD TG 412** 28-day inhalation study in rats with a 14-day recovery period.~~

Commented [ST60]: Recommend deleting, if there are concerns for effects in the respiratory tract consistent with the surfactant AOP, they will show up in the 5-day inhalation study.

**Modifications to all of the above studies should (if measureable) include pulmonary function testing, analysis of BALF, LDH release, blood oxygen (pO_2) content, and satellite reversibility. OECD TG 412 and OECD GD 39 should be consulted. Additionally, the sensory irritant potential can be measured using ASTM E 981 to determine reflex inhibition (Alarie et al., 2001).

Commented [KA611]: Alarie, Y., G.B. Nielsen, and M.M. Sch
bioassays for evaluation of indoor air quality.
Quality Handbook. Spengler, J.D., J.M.
J.F. McCarthy (eds.). New York: McGra
pp 23.21-23.40.

The results of the *in vivo* testing may be used for reassessing and recharacterizing the previously identified risks under the conditions of use for the new chemical substance. Depending on the outcome of the risk assessment, EPA will apply risk management actions on those conditions of use that result in findings of unreasonable risk, whereas no restrictions would be applied on the conditions of use where the MOEs exceed the benchmark MOE.

CONCLUSIONS

[To be added once text is finalized]

ASSOCIATED CONTENT

(Word Style "TE_Supporting_Information"). **Supporting Information**. A listing of the contents of each file supplied as Supporting Information should be included. For instructions on what should be included in the Supporting Information as well as how to prepare this material for publications, refer to the journal's Instructions for Authors.

The following files are available free of charge.

brief description (file type, i.e., PDF)

brief description (file type, i.e., PDF)

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript. ‡These authors contributed equally.

Funding Sources

Any funds used to support the research of the manuscript should be placed here (per journal style).

Notes

Disclaimer: The views expressed in this article are those of the authors and do not necessarily represent the views or policies of their respective employers. Mention of trade names or commercial products does not constitute endorsement for use.

ACKNOWLEDGMENT

Generally, the last paragraph of the paper is the place to acknowledge people, organizations, and financing (you may state grant numbers and sponsors here).

Message

From: Osman-Sypher, Sahar [Sahar_Osman-Sypher@americanchemistry.com]
Sent: 7/24/2020 12:28:23 PM
To: Stedeford, Todd [Stedeford.Todd@epa.gov]
CC: Henry, Tala [Henry.Tala@epa.gov]; Irwin, William [Irwin.William@epa.gov]; Salazar, Keith [Salazar.Keith@epa.gov]
Subject: General Surfactants Manuscript Draft - July 23 Version 4
Attachments: draft manscript general surfactants - 23 July 2020.ver.4.docx

Todd – I received minor edits from Rick Becker to address the comments from ScitoVation. Ex. 5 Deliberative Process (DP) and and changed filename to July 23, Version 4. I will circulate this version to the team to review.

Mike/Wayne are working on Ex. 5 Deliberative Process (DP) and I should have an updated draft circulated later today.

Sahar

From: Stedeford, Todd [mailto:Stedeford.Todd@epa.gov]
Sent: Friday, July 24, 2020 6:43 AM
To: Osman-Sypher, Sahar <Sahar_Osman-Sypher@americanchemistry.com>
Cc: Henry, Tala <Henry.Tala@epa.gov>; Irwin, William <Irwin.William@epa.gov>; Salazar, Keith <Salazar.Keith@epa.gov>
Subject: RE: General Surfactants Manuscript Draft - July 23 Version 2 and Associated Tables/Figure

Here is a revised draft. Ex. 5 Deliberative Process (DP)

Ex. 5 Deliberative Process (DP)

From: Osman-Sypher, Sahar <Sahar_Osman-Sypher@americanchemistry.com>
Sent: Thursday, July 23, 2020 12:03 PM
To: Stedeford, Todd <Stedeford.Todd@epa.gov>
Cc: Henry, Tala <Henry.Tala@epa.gov>; Irwin, William <Irwin.William@epa.gov>; Salazar, Keith <Salazar.Keith@epa.gov>
Subject: General Surfactants Manuscript Draft - July 23 Version 2 and Associated Tables/Figure
Importance: High

Todd:

Attached is the latest version of the manuscript (July 23, Version 2) with discussions from the call incorporated. I've also added the updated tables and tiered testing figure.

Regards, Sahar

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Surfactants Category: The Application of New Approach Methodologies (NAMs) for Assessing Inhalation Risks under the Amended Toxic Substances Control Act

Commented [HT1]: Should intro have a bit more related to exposure? And how to fit in the irritation/corrosion properties of surfactants relative to inhalation?

Tala R. Henry[a,‡], *Keith Salazar*[b,‡], *Michael P. Hayes*[c], *Wayne Kennedy*[d], *Athena M. Keene*[d], *Annie Jarabek*[e], *Stefan Moors*[f], *Lela Jovanovich*[g], *Raphael Tremblay*[c], *Ann Tveit*[f], *Richard A. Becker*[h], *Sahar Osman-Sypher*[h], *Patrick D. McMullen*[i], *Scott D. Slattery*[i], *William Irwin*[b], *Marc Odin*[j], *Julie Melia*[j], *and Todd Stedeford*[a,*]

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KEYWORDS (Word Style "BG_Keywords"). If you are submitting your paper to a journal that requires keywords, provide significant keywords to aid the reader in literature retrieval.

ABSTRACT

[To be added after co-authors feedback] The abstract should briefly state the problem or purpose of the research, indicate the theoretical or experimental plan used, summarize the principal findings, and point out major conclusions. Abstract length is one paragraph.

INTRODUCTION

The Toxic Substances Control Act (TSCA) is the primary chemicals management law in the United States and was enacted to ensure the protection of health and the environment against unreasonable risks of injury from chemical substances. In 2016, the Frank R. Lautenberg Chemical Safety for the 21st Century Act (Pub. L. 114-182; hereinafter the "Lautenberg amendments") was signed into law, thereby amending TSCA. The Lautenberg amendments included substantial changes to EPA's

authorities and responsibilities under TSCA, including requirements on EPA to make determinations on new chemical substances for unreasonable risk, sufficiency of information with determining risk, and exposure-based risk determinations. The amended TSCA also included provisions mandating the reduction and replacement of vertebrate animals in testing, to the extent practicable and scientifically justified, in support of making a determination of unreasonable risk for new and existing chemical substances. TSCA section 4(h) also charges EPA with encouraging and facilitating:

> (1) the use of scientifically valid test methods and strategies that reduce or replace the use of vertebrate animals while providing information of equivalent or better scientific quality and relevance that will support regulatory decisions under TSCA;
> (2) the grouping of 2 or more chemical substances into scientifically appropriate categories in cases in which testing of a chemical substance would provide scientifically valid and useful information on other chemical substances in the category; and
> (3) the formation of industry consortia to jointly conduct testing to avoid unnecessary duplication of tests, provided that such consortia make all information from such testing available to the Administrator.

The present investigation advances each of these TSCA mandates for chemical substances characterized as surfactants.

A surfactant is a substance that reduces the surface tension of a liquid in which it is dissolved. They are surface-active, amphiphilic compounds that self-assemble to form micelles or aggregates above a critical concentration, referred to as the critical micelle concentration (CMC). These substances are commonly used in occupational settings, in consumer products (*e.g.*,

household cleaning products, personal care products, *etc*.), and in biological research and development (R&D) as detergents, wetting agents, emulsifiers, foaming agents, and dispersants. Their use in such applications provide pathways of exposure by which potential toxicity of these compounds may occur to human or environmental receptors. Specifically, the inherent properties of surfactants may induce toxicity if exposures occur such that they can interfere with biological surfactants or tissues. For example, sodium dodecyl sulfate, a strong anionic surfactant, is used in R&D applications at concentrations up to 10% to disrupt cell membranes and to denature proteins, whereas octylphenoxypolyethoxyethanol, a mild nonionic surfactant, is used in R&D applications up to 1% to disrupt cell membranes, while preserving proteins for isolation (Burden, 2012).

Hazard concerns for surfactants were historically focused on their observed environmental effects and potential toxicity to aquatic organisms (Cowan-Ellsberry, 2014). For example, the U.S. Environmental Protection Agency (EPA) established chemical categories for cationic (quaternary ammonium) and anionic surfactants based on environmental toxicity concerns (EPA, 2010). Surfactants may also be a potential hazard concern to humans, depending on the use and route of exposure, because they can disrupt the normal architecture of the lipid bilayer and reduce the surface tension, thereby solubilizing cell membranes. For example, mucous membranes are particularly sensitive to the surface-active effects of surfactants, which have been shown to cause irritancy and injury to the eye, based on their ability to "readily penetrate the sandwiched aqueous and lipid barriers of the cornea" (Fox and Boyes, 2008).

Depending on the conditions of use, inhalation exposures to workers and/or consumers may be possible that warrant consideration in quantitative risk assessments. As noted, surfactants may cause adverse effects on mucous membranes, including the respiratory tract, and have been shown to interfere with the natural pulmonary surfactants, resulting in reduced oxygen content of arterial blood (*i.e.*, impaired gas exchange in the lung), increases in pulmonary extravascular water volume and wet-to-dry weight ratio of the lungs, grossly visible pulmonary edema, and atelectasis (Nieman and Bredenberg, 1985; Wang et al., 1993; Modell et al., 1969). However, the chemical space for surfactants that may present inhalation hazards has not been previously defined, and the potential for inhalation toxicity ranges by orders of magnitude, such as Octoxynol 9, a nonionic surfactant (Triton-X 100; CASRN 9002-93-1; 14-day lowest-observed-adverse-effect concentration [LOAEC] of 5.3 mg/m^3) (EPA, 2016; ECHA, 2020), versus didecyldimethyl ammonium chloride, a cationic surfactant and biocide (DDAC, CASRN 7173-51-5; 4-week lowest-observed-adverse-effect concentration [LOAEC] of 0.08 mg/m^3 for portal-of-entry effects) (MDEQ, 2003; CIR, 2003; ECHA, 2020).

The purpose of the present investigation was to: (1) perform a systematic review of the literature with the aim of defining the chemical space for surfactants; (2) identify appropriate toxicological analogues, when available, for identifying potential inhalation hazards and when data allow, identifying quantitative point(s) of departure for use in an inhalation risk assessment; (3) describe scientifically sound new approach methodologies (NAMs) to reduce or replace animal testing, where possible; and (4) establish a tiered-testing strategy, that utilizes NAMs, as appropriate, for new chemistries in the surfactant space.

MATERIALS AND METHODS

Systematic Literature Review

Commented [OS2]: Todd to summarize and move the details to an appendix

Objective

The objective of the literature search, screening, and retrieval process was to obtain studies that evaluated the toxicity of surfactants in the lower respiratory tract (LRT or thoracic region; *i.e.*, tracheobronchial and pulmonary regions) in exposed humans, investigated LRT outcomes in laboratory animals, or informed an adverse outcome pathway or mode of action for these agents at a cellular level (*i.e.*, *in vitro* studies). Because a list of surfactants with Chemical Abstracts Service Registry Numbers (CASRNs) was not known *a priori*, the initial PubMed search strategy was broad, with the intention of capturing potentially relevant information on any surfactant compound. Additional search strategies were employed to obtain studies not identified by keyword searching using Medical Subject Headings (MeSH or mh) and text words (tw) in PubMed.

PubMed Search

Computerized literature searches were initially conducted in PubMed in November 2016 to obtain studies related to the toxicity of surfactants in the LRT of humans and experimental animals. The search query string is presented in Table 1.

Table 1. PubMed search strategy for lung effects of surfactants.

Database Search Date	**Query String**[a]
PubMed 11/15/2016	("surface-active agents"[mh] AND lung[mh]) AND ((detergents[mh] OR aerosols[mh] OR "pulmonary surfactants"[mh]) OR (lung diseases[mh] OR cell respiration[mh] OR surface tension[mh]))

[a] Note, an Updated Literature Search was performed in April 2018, which excluded an expanded list of MeSH, query, and text words. Further details are provided in the Supplemental Information file titled "[redacted]".

Screening methods for this search included manual screening of titles/abstracts and screening of full text articles using the PECO criteria shown in Table 2.

Table 2. PECO criteria for screening of literature search results for lung effects of surfactants.

PECO element	**Evidence**[a]
Population	Humans, laboratory animals (rats, mice, hamsters, guinea pigs, dogs, non-human primates, or other inbred mammals) and mammalian cell lines
Exposure	*In vivo* (all routes), *ex vivo* (isolated perfused lung), and *in vitro*
Comparison	Any comparison (across dose, duration, or route) or no comparison (*e.g.*, case reports without controls)
Outcomes	Any examination of: • Pulmonary effects *in vivo* or *ex vivo* studies • Cytotoxicity or alternative methods in *in vitro* studies

[a] The PECO criteria were refined and more specific in the Updated Literature Search performed in April 2018. For more details, see the Supplemental Information file titled "[redacted]".

Additional Search Strategies (Gray Literature, Tree Searching, and Literature Search)

A search of the gray literature[1] was performed in September 2018 to obtain additional information pertaining to lung effects of surfactants. Resources searched for pertinent gray literature are listed in Table 3. The chemicals and compound groups identified from the initial literature search and used for gray literature searching are listed in Table 4. Screening methods for this search included manual screening of titles/abstracts and full text reports using the PECO criteria shown above in Table 2.

Table 3. List of resources to search for gray literature.

ATSDR [HYPERLINK "http://www.atsdr.cdc.gov/toxprofiles/index.asp"]
Chemtrack [HYPERLINK "http://www.chemtrack.org/White/CMR.pdf"]
CIR [HYPERLINK "http://www.cir-safety.org/ingredients"]
ECETOC publications [HYPERLINK "http://www.ecetoc.org/publications"]
ECHA [HYPERLINK "http://echa.europa.eu/web/guest/information-on-chemicals/registered-substances"]
EFSA (European Food Safety Authority) [HYPERLINK "http://www.efsa.europa.eu/"]
EPA – ChemView (incl. TSCATS data) [HYPERLINK "https://chemview.epa.gov/chemview"]
EPA – HPV Hazard Characterization Documents [HYPERLINK "http://iaspub.epa.gov/oppthpv/hpv_hc_characterization.get_report?doctype=2"]

[1] Gray literature, as used herein, has the same meaning as defined by EPA (2018) and "refers to sources of scientific information that are not formally published and distributed in peer-reviewed journal articles. These references are still valuable and consulted in the TSCA risk evaluation process. Examples of gray literature are theses and dissertations, technical reports, guideline studies, conference proceedings, publicly-available industry reports, unpublished industry data, trade association resources, and government reports."

Table 3. List of resources to search for gray literature.

EPA – HPV Risk-Based Prioritization Documents (RBPs) [HYPERLINK "http://iaspub.epa.gov/oppthpv/hpv_hc_characterization.get_report?doctype=1"]
EPA – HPVIS via ChemID - [HYPERLINK "https://chem.nlm.nih.gov/chemidplus/chemidlite.jsp"]
EPA – TSCATS 1 (available via Toxline)
EPA – pesticides - [HYPERLINK "https://iaspub.epa.gov/apex/pesticides/f?p=CHEMICALSEARCH:1"] Archive [HYPERLINK "https://archive.epa.gov/pesticides/reregistration/web/html/status.html"]
FDA [HYPERLINK "https://www.fda.gov/default.htm"]
HERA [HYPERLINK "http://www.heraproject.com/RiskAssessment.cfm"]
HSDB [HYPERLINK "http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB"]
INCHEM (CICADS, EHC, HSG, IARC, IPCS, JECFA, SIDS) [HYPERLINK "http://www.inchem.org/"]
JECDB (Japan Existing Chemical Data Base) [HYPERLINK "http://dra4.nihs.go.jp/mhlw_data/jsp/SearchPageENG.jsp"]
NICNAS http://www.nicnas.gov.au/
NITE [HYPERLINK "http://www.safe.nite.go.jp/jcheck/search.action?request_locale=en"]
NTP [HYPERLINK "https://ntpsearch.niehs.nih.gov/home"]
OECD [HYPERLINK "http://www.echemportal.org/echemportal/page.action?pageID=9"]
OECD/SIDS [HYPERLINK "http://webnet.oecd.org/hpv/ui/SponsoredChemicals.aspx"]

Table 3. List of resources to search for gray literature.

ATSDR = Agency for Toxic Substances and Disease Registry; CICADS = Concise International Chemical Assessment Document; CIR = Cosmetic Ingredient Review; ECETOC = European Centre for Ecotoxicology and Toxicology of Chemicals; ECHA = European Chemicals Agency; EFSA = European Food Safety Authority; EHC = Environmental Health Criteria; EPA = Environmental Protection Agency; FDA = Food and Drug Administration; HERA = Human and Environmental Risk Assessment; HPV = High Production Volume; HPVIS = High Production Volume Information System; HSDB = Hazardous Substances Data Bank; HSG = Health and Safety Guideline; IARC = International Agency for Research on Cancer; INCHEM = Internationally Peer Reviewed Chemical Safety Information; IPCS = International Programme on Chemical Safety; JECDB = Japan Existing Chemical Data Base; JEFCA = Joint Expert Committee on Food Additives; NICNAS = National Industrial Chemicals Notification and Assessment Scheme; NITE = National Institute of Technology and Evaluation; NTP =National Toxicology Program; OECD = Organisation for Economic Cooperation and Development; SIDS = Screening Information Data Set; TSCATS = Toxic Substances Control Act Test Submissions

Table 4. Surfactants, constituent names, and CASRNs to use for searching gray literature.

Chemical Group or Constituent Name	**CASRN**
Alkoxysilane resins	Not applicable; chemical group term
Defomaire	No data
Alevaire OR tyloxapol	25301-02-4
Triton X-100 OR polyethylene glycol p-isooctylphenyl ether	9002-93-1
Dioctyl sodium sulfosuccinate (DOSS) or butanedioic acid, 2-sulfo-, 1,4-bis(2-ethylhexyl) ester, sodium salt (1:1)	577-11-7
Polyoxyethylene-10-oleyl ether (C18:1E10)	9004-98-2
Polyoxyethylene-10-dodecyl ether (C12E10)	6540-99-4
N,N-dimethyl-dodecylamine-N-oxide (C12AO)	1643-20-5

The reference lists of the primary studies and review articles identified by the PubMed search were manually screened to identify additional pertinent literature for lung effects of surfactants (*i.e.*, tree searching). An Updated Literature Search was performed in April 2018. The details of